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Item 5 (c) (iv) of the provisional agenda*

Technical work: review of notifications of final regulatory action: chlorpyrifos

Chlorpyrifos: supporting documentation provided by Sri Lanka

Note by the Secretariat

As is mentioned in the note by the Secretariat on chlorpyrifos: notifications of final regulatory action (UNEP/FAO/RC/CRC.19/8), the annex to the present note sets out documentation provided by Sri Lanka to support its notification of final regulatory action for chlorpyrifos in the pesticide category. The present note, including its annex, has not been formally edited.

* UNEP/FAO/RC/CRC.19/1/Rev.1.

Annex

Chlorpyrifos: supporting documentation provided by Sri Lanka

List of documents:

1. Ban of registration by the Government Extraordinary Gazette No. 1999/33 dated 28/12/2016 under the Control of Pesticides Act No.33 of 1980.
2. USEPA Regulatory Report (Human Health Risk Assessment Chlorpyrifos Phase 4, U.S. Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division (7509C) Deborah C. Smegal, M.P.H., Risk Assessor, June 8, 2000).
3. Exposure and health risk assessment for farmers occupationally exposed to chlorpyrifos in Sri Lanka; and drinking water and house dust analysis for chlorpyrifos. Aponso *et al*., (2002). Annals of the Sri Lanka Department of Agriculture, 2002 4: 233–244.
4. Analysis of water for pesticides in two major agricultural areas of the dry zone by Aponso *et al.* (2003) Annals of the Sri Lanka Department of Agriculture, 2003 5: 7–22.
5. Seasonal exposure of fish to neurotoxic pesticides in an intensive agricultural catchment, Uma-Oya, Sri Lanka: Linking contamination and acetylcholinesterase inhibition. Sumith et. al (2012). Environmental Toxicology and Chemistry, Vol. 31, No. 7, pp. 1501–1510, 2012.
6. Assessment of Health Risk in Human Populations Due to Chlorpyrifos. Marasinghe *et. al* (2014). Toxics 2014, 2, 92-114.
7. Chlorpyrifos contamination of fresh water in a commercial vegetable cultivation area in Sri Lanka and factors affecting contamination. Menike *et al* (2012). J.Natn.Sci.Foundation Sri Lanka 2012 40 (4): 333-344.
8. Do Targeted Bans of Insecticides to Prevent Deaths from Self-Poisoning Result in Reduced Agricultural Output?. Manuweera et. al (2008). Vol. 116, No. 4, April 2008 - Environmental Health Perspectives.
9. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Eddleston et. al (2005). Lancet 2005 Oct; 366(9495):1452-9.
10. EXTOXNET Extension Toxicology Network Pesticide Information Profiles – Chlorpyrifos. http://extoxnet.orst.edu/pips/chlorpyr.htm.

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PART I : SECTION (I) — GENERAL

Government Notifications

L.D.B. 5/83

CONTROL OF PESTICIDES ACT, No. 33 OF 1980

Order under Section 11

BY virtue of the powers vested in me by Section 11 of the Control of Pesticides Act, No. 33 of 1980, I, Jayakody Arachchige Sumith, Registrar of Pesticides, do by this Order, in the interest of the public and on the advice of the Pesticides Technical and Advisory Committee, cancel every licence issued in respect of pesticides containing the active ingredients bearing the Chemical Abstract Service Registry Numbers specified in the Schedule hereto.

Dr. J. A. Sumith,
Registrar of Pesticides.

Peradeniya,
19th December 2016.

SCHEDULE

Chemical Abstract Service Registry Number	*Active Ingredient*
1563-66-2	Carbofuran
63-25-2	Carbaryl
2921-88-2	Chlorpyriphos

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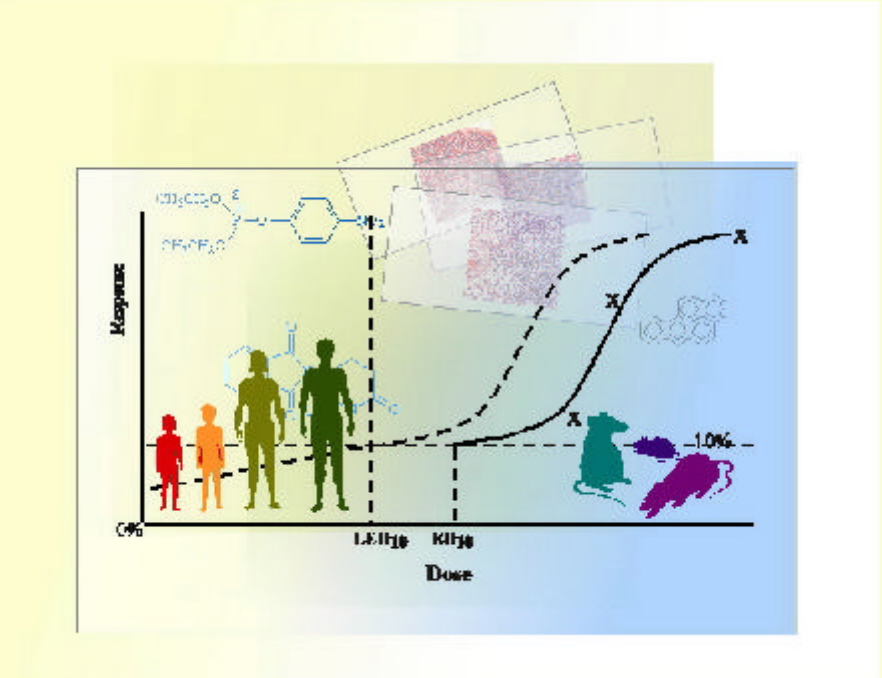
HUMAN HEALTH RISK ASSESSMENT

CHLORPYRIFOS



U.S. Environmental Protection Agency
Office of Pesticide Programs
Health Effects Division (7509C)

Deborah C. Smegal, M.P.H., Risk Assessor
June 8, 2000

HUMAN HEALTH RISK ASSESSMENT

CHLORPYRIFOS

Phase 4

Risk Assessment Team:

Lead Risk Assessor:	Deborah C. Smegal, M.P.H., Toxicologist
Dietary Risk:	David Soderberg, Chemist
Residue Chemistry:	Steven Knizner, Senior Scientist/Chemist
Product Chemistry:	Steven Knizner, Senior Scientist/Chemist
Agricultural, Occupational and Residential Exposure:	Timothy Leighton, Environmental Health Scientist
	Deborah C. Smegal, M.P.H., Toxicologist
Toxicology:	Deborah C. Smegal, M.P.H., Toxicologist
Incident Review:	Jerome Blondell, Health Statistician
	Virginia Dobozy, Veterinary Medical Officer

Management:

Senior Scientist:	Steven Knizner
Branch Chief:	Jess Rowland
Division Director:	_____
	Margaret J. Stasikowski, June 8, 2000

Background

Attached is HED's risk assessment of the organophosphate pesticide, chlorpyrifos, for purposes of issuing a Reregistration Eligibility Decision (RED) Document for this active ingredient. Cumulative risk assessment considering risks from other pesticides or chemical compounds having a common mechanism of toxicity is not addressed in this document. This risk assessment updates the October 18, 1999 version and addresses the Public Comments in accordance with Phase 3 of the Tolerance Reassessment Advisory Committee (TRAC) Organophosphate (OP) Pilot Process. EPA and the registrants have agreed to certain modifications to the use of chlorpyrifos to mitigate dietary, worker and residential risks. This risk assessment incorporates elements of the risk mitigation agreement in a number of its analyses in order to characterize post-mitigation risks. The disciplinary science chapters and other supporting documents for the chlorpyrifos RED are also included as attachments as follows:

- Report of the Hazard Identification Assessment Review Committee. D. Smegal (4/6/2000, HED Doc No. 014088)
- Report of the FQPA Safety Factor Committee. Brenda Tarplee (4/4/00; HED Doc No. 014077)
- Revised Product and Residue Chemistry Chapter. Steven Knizner (June 2000)
- Toxicology Chapter. Deborah Smegal (4/18/00; D263892)
- Occupational/Residential Handler and Post-Application Residential/Non-Occupational Risk Assessment. D. Smegal/T. Leighton (June 2000; D266562)
- Agricultural and Occupational Exposure Assessment: Tim Leighton (June 2000; D263893)
- Acute Dietary Risk Assessment for Chlorpyrifos. (D. Soderberg June 2000, D263890)
- Chronic Dietary Exposure Assessment for Chlorpyrifos. D. Soderberg (June 2000, D263889)
- Chlorpyrifos Incident Review Update: Jerome Blondell (4/20/00).
  Update of Incident Data on Chlorpyrifos for Domestic Animals. Virginia Dobozy (04/26/99; D255514)
- Analysis of Chlorpyrifos IDS Data for Domestic Animals. Virginia Dobozy (1/23/95)
- Drinking Water Assessment from the Environmental Fate and Effects Division (EFED). Michael Barrett (11/13/98)

‘ EFED Concerns over well contamination associated with termiticide use and EFED Recommended Concentrations for HED Drinking Water Assessment of Chlorpyrifos. Henry Nelson (10/6/99)

‘ Chlorpyrifos Preliminary Risk Assessment for Trichlorpyridinol (TCP) Metabolite. S. Knizner. D265035.

HED’s Hazard Identification Assessment Review Committee (HIARC) reviewed the toxicological database for chlorpyrifos and selected toxicological endpoints for acute oral, chronic oral and for short-, intermediate and long-term dermal and inhalation exposure risk assessment in February 1999, and January 2000 (memorandum dated April 6, 2000). HED’s FQPA Safety Factor Committee reviewed the hazard and exposure data for chlorpyrifos on January 24, 2000, and deferred to the Office of Pesticide Programs Division Directors and senior scientists (DD-SS). The DD-SS recommended that the 10X FQPA Safety Factor (as required by Food Quality Act of August 3, 1996) be retained in assessing the risk posed by this chemical (memorandum dated April 4, 2000).

In June 1997, the registrants of chlorpyrifos voluntarily agreed to measures designed to reduce household exposure to chlorpyrifos, as part of a risk reduction plan. This voluntary plan included deletion of indoor broadcast use, use as an additive to paint, direct application to pets (sprays, shampoos and dips), and indoor total-release foggers. The technical chlorpyrifos products have been amended to reflect the negotiated plan. The technical label limits end use product labeling to only those sites which are specified on its label. In addition, the registrants have implemented measures to improve education, training, and labels, and report and analyze incidents. In addition, as part of this agreement, the registrants agreed to work with EPA to develop broad, market-wide policies for all indoor insecticides for a number of areas.

EPA and the registrants have agreed to certain modifications to the use of chlorpyrifos to mitigate dietary, worker and residential risks. This risk assessment incorporates elements of this agreement in a number of its analyses in order to characterize post-mitigation risks. The agreement includes:

‘ Agricultural Uses

- Restrict use on apples to pre-bloom (dormant) application only
- Cancel use on tomatoes
- Implement revised restricted-entry intervals for all agricultural crops.

‘ Non-Agricultural Uses

- Cancel all indoor residential uses (except fully contained ant baits in child resistance packaging).
- Cancel all outdoor residential uses (except limited public health uses).
- Cancel all indoor and outdoor non-residential uses (e.g. FHE) except:
- Use on golf courses
- Limited public health uses
- Limited use in industrial settings (e.g. manufacturing plants, ship holds)
- Cancel whole house “post-construction” termiticide use.
- Phase out limited post-construction spot and local termiticide treatments
- Phase out pre-construction termiticide treatments
- Reduce the maximum application rate for phased-out termiticide treatments to a 0.5% concentration.
- Reduce the maximum application rate for use on golf courses to 1 lb. active ingredient per acre.

In addition to these agreed upon actions the Agency will also propose to revoke the tolerance on tomatoes and reduce the tolerances on apples and grapes to 0.01 ppm. These changes were also included in the analysis of post-mitigation dietary exposure.

CHLORPYRIFOS REVISED RISK ASSESSMENT

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CHLORPYRIFOS

1.0 Executive Summary

Background

The Health Effects Division (HED) has conducted a Human Health Risk Assessment for the active ingredient chlorpyrifos for the purposes of making a reregistration eligibility decision (RED). The toxicological database is complete and adequate to support reregistration in accordance with the Subdivision F Guidelines for a food use chemical. Residue chemistry requirements are substantially complete pending receipt of limited confirmatory data.

Chlorpyrifos, [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate], is a broad-spectrum, chlorinated organophosphate insecticide that was first registered in 1965 to control foliage- and soil-borne insect pests on a variety of food and feed crops. Chlorpyrifos' most common trade names are Dursban, Empire 20, Equity, and Whitmire PT 270. Lorsban is a trade name for agricultural-use products. It is one of the most widely used organophosphate insecticides in the U.S., and is one of the major insecticides used in residential settings. Approximately 21 to 24 million pounds are used annually in the U.S, of which approximately 11 million pounds are applied in non-agricultural settings. There are approximately 800 registered products containing chlorpyrifos on the market. Registered uses include: variety of food crops (i.e., there are approximately 112 tolerances for food/feed commodities); turf and ornamental plants; greenhouses; sodfarms; indoor pest control products (e.g., crack and crevice); structural pest control (e.g., termites); and pet collars. It is registered for use in residential and commercial buildings, schools, daycare centers, hotels, restaurants and other food-handling establishments, hospitals, stores, warehouses, food manufacturing plants, vehicles, and livestock premises. In addition, it is used as a mosquitocide, and as impregnated in ear tags for cattle. In 1998, Dow AgroSciences (DAS) estimated that 70% of the urban chlorpyrifos use involved termite control. Chlorpyrifos products are widely used by homeowners and professionals.

The following are formulation types for chlorpyrifos: wettable powder, emulsifiable concentrate, dust, granular, bait, flowable concentrate, impregnated material, pelleted/tableted, pressurized liquid, and microencapsulated. Dry flowable and wettable powder formulations in open bags are no longer supported by the primary registrant, Dow AgroSciences (DAS). Therefore, these formulations are not assessed in this risk assessment and are not eligible for re-registration.

Hazard

Chlorpyrifos is moderately toxic following acute oral, dermal and inhalation exposures (toxicity category II). Chlorpyrifos affects the nervous system by reversibly inhibiting the activity of cholinesterase (ChE), an enzyme necessary for the proper functioning of the nervous system. Inhibition of ChE is the most sensitive effect in all animal species evaluated and in humans, regardless of route or duration of exposure. In animals, significant inhibition of plasma and red blood cell (RBC) ChE occur at doses below those that cause brain ChE inhibition. Data from two human studies suggest that humans are similarly and possibly more sensitive than animals following acute and short-term oral exposure and acute dermal exposure based on plasma ChE inhibition and/or possible clinical signs. Females are slightly more sensitive than males based on ChE inhibition and acute toxicity (comparison of LD_{50}'s). Studies in the scientific literature suggest that neonates are more sensitive to oral chlorpyrifos exposure than adults for ChE inhibition and behavioral effects. The increased sensitivity of the young may be attributed to a reduced capacity to detoxify chlorpyrifos.

Developmental and reproductive effects have been observed in rats, rabbits and/or mice, but only at doses that induced maternal or parental toxicity. In rats, chlorpyrifos causes delayed alterations in brain development in offspring of exposed mothers. Several studies in the peer reviewed literature and results of the guideline developmental neurotoxicity study are supportive of the possibility that chlorpyrifos exposure may affect brain development (e.g., altered synaptic development, alterations in DNA, RNA, and protein synthesis, inhibition of mitosis and mitotic figures, and disruption of the structural architecture of the brain). There are suggestive data that these effects may arise independent of cholinesterase inhibition.

Chlorpyrifos did not induce treatment-related tumors or provide evidence of carcinogenicity in two chronic rat or two chronic mouse studies. Chlorpyrifos was not mutagenic in bacteria, or mammalian cells, but did cause slight genetic alterations in yeast and DNA damage to bacteria.

For the purposes of this assessment, HED has concluded that the primary metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), is not of toxicologic concern because 3,5,6-TCP does not induce cholinesterase inhibition (58 FR 19354, April 14, 1993). However, because of potential exposure to TCP in food and residential settings, and evidence of increased susceptibility of rabbit fetuses relative to dams based on the DAS-submitted rabbit developmental study, HED conducted a screening-level risk assessment for TCP. This assessment is attached in memorandum from S. Knizner to D. Smegal, D265035 June 5, 2000.

The toxicity endpoints used in this document to assess hazards include acute dietary and chronic dietary reference doses (RfDs), and short-, intermediate- and long-term dermal and inhalation doses. In light of the developing Agency policy on use of toxicology studies employing human subjects, HED selected doses and endpoints for risk assessment based solely on animal studies. Therefore, this document contains risk

assessments based on animal toxicity studies.

The acute dietary RfD of 0.005 mg/kg/day is based on a no-observed adverse effect level (NOAEL) of 0.5 mg/kg/day from an acute oral rat blood time-course study that observed 28-40% plasma cholinesterase (ChE) inhibition 3-6 hours after dosing male rats with a single dose of 1 mg/kg/day (the lowest-observable adverse effect level, LOAEL). This NOAEL is supported by statistically significant 30% RBC ChE inhibition 4 hours after a single 1.5 mg/kg/day exposure by a study in the scientific literature (Zheng et al. 2000). The chronic RfD of 0.0003 mg/kg/day is based on an oral NOAEL of 0.03 mg/kg/day for significant plasma and red blood cell (RBC) ChE inhibition at 0.22 to 0.3 mg/kg/day (LOAEL) based on a weight of the evidence consideration of 5 toxicity studies in dogs and rats. An uncertainty factor of 100 (10X for interspecies extrapolation and 10X for intraspecies variability) was applied to the NOAELs to obtain the RfDs.

A route-specific short-term dermal NOAEL of 5 mg/kg/day from a 21-day dermal rat study has been selected based on plasma and RBC ChE inhibition of 45% and 16%, respectively at 10 mg/kg/day (LOAEL). A dermal absorption adjustment is not necessary because a dermal study was selected. The intermediate- and long-term dermal NOAELs and long-term inhalation NOAEL are 0.03 mg/kg/day based on statistically significant plasma and RBC ChE inhibition that occurred at 0.22 to 0.3 mg/kg/day based on a weight of the evidence of 5 toxicity studies in dogs and rats. Because an oral NOAEL was selected, a 3 percent dermal absorption factor was used. Dermal absorption was estimated to be 3 percent based on the ratio of the oral LOAEL of 0.3 mg/kg/day from the rat developmental neurotoxicity (DNT) study to the dermal LOAEL of 10 mg/kg/day from the 21-day rat dermal study. This absorption factor is comparable to the dermal absorption estimated from human data of 1-3%.

The short- and intermediate-term inhalation NOAEL is 0.1 mg/kg/day from two separate 90-day rat inhalation studies that did not observe effects at the highest vapor concentration tested. HED selected a LOAEL of 0.3 mg/kg/day for 43% plasma and 41% RBC ChE inhibition from the oral developmental neurotoxicity study in rats to complete the dose-response assessment. A 100% default inhalation absorption factor (i.e., inhalation and oral absorption are equivalent) was used.

FQPA Safety Factor

The Food Quality Protection Act (FQPA) Safety Factor Committee re-evaluated the previous FQPA safety factor recommendation based on new hazard information, and deferred to the OPP Division Directors and several Agency senior scientists (DD-SS group) for the recommendation. The Division Directors and senior scientists (DD-SS group), recommended that the FQPA safety factor should be **retained at 10X** for the protection of infants and children from exposure to chlorpyrifos. The FQPA safety factor is applicable to **females 13-50, and infants and children** population subgroups for acute and chronic dietary risk assessments and residential and other non-occupational risk assessments of all durations. The safety factor was retained because new data in the

literature (Zheng et al. 2000) demonstrated increased neonatal sensitivity following a low-level single oral exposure, and a registrant submitted developmental neurotoxicity (DNT) study showed a clear qualitative difference in response (i.e., susceptibility) between adult rats and their offspring. Cholinesterase inhibition was observed in dams versus structural effects in the developing brain of the offspring.
In addition, the new data in the literature also gave rise to uncertainties such as the suggestion that the inhibition of cholinesterase may not be essential for adverse effects on brain development; and the lack of an offspring NOAEL in the DNT based upon structural alterations in brain development as the toxicity endpoint of concern (i.e., effects were seen at the lowest dose evaluated).

Dietary Exposure and Risk

HED conducted the most highly refined acute probabilistic and chronic deterministic dietary (food) exposure analyses possible using the Dietary Exposure Evaluation Model (DEEM). Both the acute and chronic dietary analyses incorporate monitoring data obtained from U.S. Department of Agriculture's (USDA's) Pesticide Data Program (PDP), the Food and Drug Administration's (FDA's) Surveillance Monitoring Program, in addition to monitoring data from Dow AgroSciences' (DAS')1993 National Food Survey (NFS) (a market basket survey), and field trial data for a limited number of crops. Percent crop treated data and processing and cooking factors were also used to refine the exposure estimates. The Agency's acute and chronic analyses incorporated PDP and FDA monitoring data to the greatest extent possible, and NFS data for seven of the nine commodities included in the survey (milk, apple juice, applesauce, orange juice, ground beef, pork sausage and peanut butter). The NFS data for fresh apples were also included in a sensitivity analysis. The NFS tomato data were not included because only 54 samples were collected from Florida, while more extensive and recent data for fresh tomatoes are available from PDP (881 samples, collected in 1996 and 1997). PDP monitoring data also reflect the use of chlorpyrifos on imported fresh tomatoes (a significant source of fresh tomatoes). Therefore the PDP fresh tomato residue data were used exclusively in all analyses.

Three data sets are available for estimating residues on fresh apples: PDP data for analysis of individual single apples; PDP “decomposited” apple data; and NFS “decomposited” apple data. Use of each of these three data sets for fresh apples leads to a different exposure estimate. The dietary exposure analysis has been performed using all commodities having chlorpyrifos uses and each of the apple data sets separately: PDP data for single apples; PDP “decomposited” apple data; and NFS “decomposited” apple data.

In both acute and chronic risk assessments, exposure was compared to a population adjusted dose, (PAD), which is the reference dose (RfD) reflecting retention of the FQPA 10x factor for females and children. HED considers dietary residue contributions greater than 100% of the PAD to be of concern. The acute and chronic PADs are 0.0005 and 0.00003 mg/kg/day, respectively for children and females 13-50 years. The acute and chronic PADs are 0.005 and 0.0003 mg/kg/day, respectively for all

other population groups. The Agency's highly refined **acute dietary exposure** estimates at the 99.9th percentile were greater than 100% of the aPAD for all child subpopulations based on the 1999 PDP single apple data, the decomposited 1994-1997 PDP apple data, and/or the decomposited 1993-1994 NFS apple data. Children 1-6 years old were the most highly exposed population subgroup, regardless of which data set is used for fresh apples. Apples contribute most to the child risk estimates. For **children 1-6 years** old, risk estimates ranged from **170% to 355% of the aPAD** depending on which fresh apple data set was used. Use of PDP's 1999 single apple data resulted in the highest exposure estimates. Use of the decomposited NFS fresh apple data resulted in the lowest exposure estimates. Because the PDP single apple data are the most recent and do not require decompositing, these data are expected to provide the most reliable exposure and risk estimates. However, no matter which of the three data sets is used for fresh apples, the critical exposure commodity (CEC) analysis indicated that residues on fresh apples were the major contributor to dietary exposure estimates for children 1-6 years old at the 99.9th percentile exposure. Residues on whole tomatoes and grapes were the next major contributors to exposure.

Various risk mitigation measures were examined to reduce acute dietary exposure and risk estimates. Risk estimates could be reduced to less than 100% of the aPAD for children 1-6 years old only with mitigated exposures from consumption of fresh apples, grapes and tomatoes. Acute dietary risk estimates for children 1-6 years old were reduced to 82% of the aPAD based on the following mitigation measures: reduction of the apple tolerance to 0.01 ppm based on dormant application only; reduction of the grape tolerance to 0.01 ppm based on the domestic use pattern; and deletion of the use and removal of the tolerance on tomatoes. Ingestion of residues detected on a number of commodities (spinach, squash and carrots) that lack chlorpyrifos tolerances does not impact the acute dietary risk estimates. Because chlorpyrifos is not registered for use on these crops, these residues represent chlorpyrifos misuse or possibly spray drift.

The Agency's average **chronic dietary exposure** estimates for the U.S. population and all subgroups, with or without consideration of food handling establishment use, **are below HED's level of concern.** Without consideration of the food handling establishment (FHE) use, the average exposure estimates comprised 3% of the cPAD for the general population and 61% of the cPAD for the most highly exposed subgroup, children 1-6 years old. The Agency average exposure estimates including the food handling establishment use comprised 4% of the cPAD for the general population and **81% of the cPAD** for the most highly exposed subgroup, children 1-6 years old. The risk mitigation measures designed to reduce acute dietary risk also reduce chronic dietary risk. Children 1-6 years old remain the most highly exposed subpopulation, with risk estimates of 51% and 31% of the cPAD, including the FHE use or using zero residues for the FHE use, respectively. Ingestion of residues on a number of commodities (spinach, squash and carrots) that lack chlorpyrifos tolerances does not impact the chronic dietary risk estimates.

Drinking Water Exposure and Risk

The available environmental fate data suggest that chlorpyrifos has a low potential to leach to groundwater in measurable quantities from typical agricultural uses, however, there have been instances of well contamination following termiticide use. The available data indicate that the primary metabolite of chlorpyrifos, 3,5,6-TCP is more mobile, and significantly more persistent in many soils, especially under anaerobic conditions. The Agency has provided a screening-level drinking water assessment based on simulation models and an analysis of available monitoring data to estimate the potential concentrations of chlorpyrifos in ground and surface water.

The Agency conducted an analysis of over 3000 filtered groundwater monitoring well data available in U.S. Geological Survey's National Water Quality Assessment (NAWQA) Program databases, and in the Agency's Pesticides in Ground Water Data Base (PGWDB). Chlorpyrifos was infrequently detected in groundwater (< 1% of the 3000 wells), with the majority of concentrations reported to be <0.01 ppb, and a maximum detected concentration of 0.65 ppb in the PGWDB. Groundwater concentrations following termiticide use are potentially much higher, with a maximum reported concentration of 2090 ppb because of well contamination. The Agency also performed screening-level model estimates of chlorpyrifos concentrations in groundwater using SCI-GROW. Inputs to the models included high exposure agricultural scenarios for major crops (alfalfa, corn, citrus, and tobacco) at the maximum application rates. The estimated concentrations of chlorpyrifos in groundwater using the SCI-GROW screening model range from 0.007 to 0.103 ppb.

The Agency also evaluated more than 3000 samples from 20 NAWQA study units for surface water. In surface water, chlorpyrifos was detected at frequencies up to 15% of 1530 agricultural streams, 26% of 604 urban stream samples in 1997 and in 65% of 57 urban stream samples from Georgia, Alabama and Florida in 1994. The maximum reported dissolved chlorpyrifos concentration in surface water is 0.4 ppb, with the 95th percentile at 0.026 ppb, and the majority of concentrations < 0.1 ppb. However, the Agency notes that the monitoring data are not available for the most vulnerable watersheds or groundwater where chlorpyrifos use is pervasive. The Agency also performed screening-level model estimates of chlorpyrifos concentrations in surface water using Tier I GENEEC or Tier II PRZM/EXAMS. Estimated maximum 90 day average and peak concentrations of chlorpyrifos in surface water using the PRZM/EXAMS screening model are 6.7 Fg/L and 40.6 ppb, respectively.

Based on the monitoring data and model estimates the Agency used a range of upper-bound estimated environmental concentrations (EECs) in water for the water assessment. For the acute and chronic groundwater assessment an EEC range of 0.007 to 0.103 ppb was used based on screening-level model estimates. For the acute surface water assessment a range of 0.026 to 0.4 ppb was used, based on the 95th percentile and maximum reported concentrations from monitoring data. For the chronic surface water assessment, the 95th percentile concentration from monitoring data of 0.026 ppb was used. For termiticide use, the Agency had upper-bound groundwater concentrations of 30 to 2090 ppb for the acute exposures, based on well remediation efforts and monitoring data, respectively, and 8.3 to 578 ppb (acute values adjusted for partial environmental

degradation) for chronic exposures. The SCIGROW model and the monitoring data do not reflect actual drinking water concentrations after dilution (from source to tap) or drinking water treatment.

HED calculated drinking water levels of comparison (DWLOCs) assuming mitigation measures for diet and residential uses. Except for possible contamination resulting from termiticide use, the acute and chronic DWLOCs are greater than the EECs and thus do not exceed HED's level of concern.

Exposures to chlorpyrifos from groundwater because of well contamination as a result of the termiticide use for either acute or chronic durations may result in exposures that are potentially of concern. However, implementation of PR-96-7 has reduced the reported incidents of groundwater contamination resulting from termiticide treatment.

Occupational and Residential Exposure and Risk

Occupational and residential exposures to chlorpyrifos can occur during handling, mixing, loading and application activities. Occupational postapplication exposure can occur for agricultural workers re-entering treated fields such as during scouting, irrigation and harvesting activities.

Residential postapplication exposure can occur following treatment of lawns, or residences for cockroaches, carpenter ants, termites, and other insects. In addition, there is a potential for inadvertent oral exposure to children from eating chlorpyrifos-treated turf and soil or licking fingers following contact with treated areas. Postapplication exposure to children can occur in locations other than the home, including schools, daycare centers, playgrounds, and parks.

There is insufficient use information and exposure data to assess exposure resulting from use in vehicles (i.e., planes, trains, automobiles, buses, boats) and other current label uses such as treatment of indoor exposed wood surfaces, supermarkets, theaters, furniture, and draperies, etc. HED has concern for these uses based on the residential scenarios assessed within this document, which show that nearly all current uses evaluated result in exposures that exceed HED's level of concern. HED has requested additional exposure data for all registered uses not evaluated in this assessment. Although there is concern for these uses, the Agency believes that exposure to these uses will not be higher than the scenarios evaluated in the risk assessment.

HED has conducted dermal and inhalation exposure assessments for: occupational and residential handlers; occupational postapplication; and residential postapplication dermal and inhalation exposure to adults and children as well as inadvertent oral exposure to children. The exposure duration for short-term assessments is defined as 1 to 30 days. Intermediate-term durations are 1 month to six months, and long-term exposures are durations greater than six months. The duration of exposure is expected to be: short-term for agricultural handlers; intermediate and long-term for the occupational handler in residential settings (i.e., lawn care operator and pest control operator); intermediate-term

for occupational postapplication; and short-term for the residential handler. The postapplication residential exposures evaluated in this assessment are considered short-term, except for exposures from termiticide treatment which is considered a long-term exposure.

For the dermal and inhalation risk assessment, risk estimates are expressed in terms of the Margin of Exposure (MOE), which is the ratio of the NOAEL selected for the risk assessment to the exposure level. For occupationally exposed workers, MOEs >100 (i.e., 10x for interspecies extrapolation and 10x for intraspecies variability) do not exceed HED's level of concern. For residential populations, MOEs >1000, which includes the 10x FQPA safety factor for females 13-50 and children, do not exceed HED's level of concern. The target MOE of 1000 is applicable for residential handlers.

The **majority of occupational risk estimates do not exceed HED's level of concern** with appropriate personal protective equipment (PPE) or engineering controls. The results of the short-term handler assessments indicate that only 1 of the 16 potential exposure scenarios did not provide at least one application rate with a total MOE(s) greater than or equal to 100 at either the maximum PPE (i.e., coveralls over long pants, long sleeved shirts, and chemical resistant gloves while using open systems) or using engineering controls (i.e., closed systems). In the majority of cases, dermal exposure contributes more significantly to the total MOE than inhalation exposure.

In total, exposure and risk estimates were calculated for 56 scenarios. Based on the maximum level of protection (i.e., various levels of PPE or engineering controls) 2 MOEs are estimated to be less than 10; 6 MOEs are between 10 and 50; 9 MOEs are between 50 and 100, and 39 MOEs are greater than 100. Fourteen of the scenarios were evaluated based on data obtained from five chemical-specific studies submitted by DAS. The agricultural handler assessments are believed to be reasonable high end exposure representations of chlorpyrifos uses.

There is insufficient information (e.g., dermal and inhalation exposure data) to assess 3 scenarios: seed treatment uses, dip applications (e.g., preplant peach root stock, and nursery stock), and dry bulk fertilizer applications to citrus orchard floors. Given the results from the other scenarios assessed, these scenarios may also need to be mitigated. HED has requested data for these scenarios.

The results of the **Pest Control Operator (PCO)/Lawn Care Operator (LCO) handler assessment in residential/recreational settings** for short-, intermediate and/or long-term exposure scenarios indicate that **most** of the MOEs are less than 100, and therefore **exceed HED's level of concern**. The only scenarios that result in MOEs above 100, and do no exceed HED's level of concern are: (1) lawn care professionals that wear PPE and mix and load liquid lawn products (but do not apply) (total MOEs 100-820), (2) workers who mix/load or apply chlorpyrifos for aerial mosquitocide applications of less than 30 days with the use of engineering controls (closed systems)(total MOEs 160-240); (3) workers who mix/load or apply chlorpyrifos for ground-based fogger mosquitocide

applications up to several months with the use of PPE or engineering controls (total MOEs 100-560), and (4) most golf course workers who use the typical rate of 1 lb ai/acre or mixer/loaders of wettable powder that handle product to treat 4 lb ai/acre for less than 30 days (total MOE 100-400).

A number of risks were estimated based on chemical-specific biomonitoring studies submitted by DAS (i.e., indoor crack and crevice treatment, broadcast turf application, and pre- and post-construction termiticide treatment) in which the LCOs/PCOs wore label-specified PPE or PPE in addition to that specified on labels. Several of these studies did not apply the product at the maximum label rate, or only evaluated exposures for a few hours (i.e. 1-3 hours) of the work day, and consequently could underestimate exposures and risks to LCOs/PCOs. Overall, the exposures and risk estimates for LCOs/PCOs based on the chemical-specific biomonitoring studies are considered to be central tendency estimates because they evaluated less than a full day's exposure at the maximum label rate. In the absence of chemical-specific data, LCO/PCO exposures were estimated using data from Pesticide Handlers Exposure Database (PHED) or the Draft Residential SOPs.

The results of the **short- and intermediate-term postapplication** assessments for **workers at agricultural use sites** indicate that restricted entry intervals (REIs) need to be established. REIs represent the duration in days which must elapse before the Agency would not have a concern (MOE $100) for a worker wearing a long-sleeved shirt and long pants to enter the treated area and perform specific tasks. The **REIs range from 24 hours** for the low, medium, and high crop grouping matrix **to 10 days** for harvesting cauliflower. In short, REIs are 24 hours for all crops except the following: cauliflower (10 days), all nut trees (2 days), all fruit trees (4 days), and citrus (5 days). The occupational postapplication assessment is believed to be reasonable high end representations of chlorpyrifos uses. Four registrant-submitted dislodgeable foliar residue (DFR) studies are included in this assessment. Specifically, data are available for sugar beets, cotton, sweet corn, almonds, pecans, apples, citrus, cauliflower, and tomatoes. The short-term MOEs for postapplication exposure for mow/maintenance workers at golf courses are above 100 (110-210) and therefore, do not exceed HED's level of concern, even at the maximum label rate of 4 lb ai/acre.

All nine short-term residential handler exposure scenarios evaluated have total dermal and inhalation MOEs (based on typical, and maximum usage rates) that **exceed HED's level of concern** defined by a target MOE of 1000. MOEs for the residential handler ranged from 3 to 900 for dermal risk, from 120 to 57,000 for inhalation risk, and from 3 to 880 for total dermal and inhalation risk. The following scenarios were evaluated: (1) indoor crack and crevice treatment, (2) lawn treatment with liquid products, (3,4,5) lawn treatment with granular formulations via push-type spreader, belly grinder and hand application, (6) application of ready to use products, (7) dust product applications, (8) paintbrush application, and (9) treatment of ornamentals. In some instances, when the product is not applied at the maximum label rate, the MOEs are above 1000 (i.e., 2 oz crack and crevice spot treatment with a MOE of 1600). Only one of the residential handler

scenarios was evaluated using chemical-specific data submitted by DAS, the remaining scenarios were evaluated using the Residential SOPs or PHED.

The results of the **residential postapplication** exposure scenarios indicate that **seven of the nine** scenarios evaluated have MOEs that are less than 1000, and therefore **exceed HED's level of concern**. These scenarios include exposures following indoor crack and crevice treatment, pet collars, termiticide treatments, liquid and granular lawn treatments and yard and ornamental sprays. In addition, for post application exposure to children following perimeter applications to homes, it was estimated that more than seven hand-to-mouth events or more than 8 minutes of play on treated turf the day of treatment could result in potential exposures that could exceed the Agency's level of concern (i.e., MOE < 1000). An additional scenario could not be quantitatively evaluated (post application exposure to insecticidal dust product use) due to an absence of chemical-specific data and recommended procedures in the residential SOPs. MOEs that exceed HED's level of concern ranged from 6 to 980 for total dermal, inhalation and inadvertent oral (in the case of children) risk. The only residential/recreational scenarios that resulted in a MOE above 1000 are the aerial and ground-based fogger adult mosquitocide application (MOEs 15,000 to 42,000) and adolescent and adult golfers for the typical application rate of 1 lb ai/acre (MOEs 1500 - 2400). Several of the residential postapplication risks were estimated based on chemical-specific studies submitted by DAS (i.e., crack and crevice treatment of the kitchen and bathroom, broadcast treatment of turf with chlorpyrifos spray or granules, and termiticide treatment). The exposure and risk estimates based on the chemical-specific studies are considered to be reasonable central-tendency estimates (i.e., arithmetic mean or median exposure was used to calculate risk). Because these studies were conducted in adults, standard EPA assumptions were used to estimate child exposures.

Poisoning Incidents

Because of its widespread use in residences, chlorpyrifos is often involved in unintentional exposures. About 6% of all pesticide-related calls (estimated at 7,000 annually) received by the poison control centers are related to chlorpyrifos. The overwhelming majority of cases experience only minor symptoms, but about 200 cases per year are serious enough to require special medical attention. Although only a small proportion of cases involve products used by pest control operators, these exposures often involve exposures to concentrated chemical, which can lead to more serious health effects.

Aggregate Exposure and Risk

As mandated by the FQPA amendments to the Federal Food, Drug and Cosmetic Act (FFDCA), the Agency must consider total aggregate exposure from food, drinking water, and residential sources of exposure to chlorpyrifos. Based on the mitigation plan, this aggregate assessment considers exposure to chlorpyrifos from food, drinking water and residential uses. In addition, the Agency has concerns about possible residential exposures from chlorpyrifos spray drift. The Agency is currently developing methods to

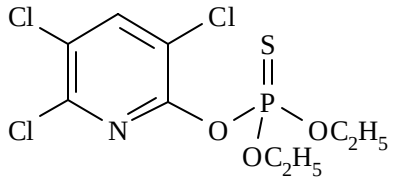
assess residential exposures from spray drift, and these will be assessed in the future when new methods are available. The **acute aggregate risk estimates do not exceed HED's level of concern** because combined exposure to chlorpyrifos through food and drinking water sources are <100% aPAD. The **short-term aggregate risk estimates do not exceed HED's level of concern** based on concurrent exposure to chlorpyrifos from golfing, mosquito abatement activities, in addition to food and drinking water. The **chronic** food and drinking water **aggregate risk estimates do not exceed HED's level of concern**.

Although not all of the risk estimates for termiticide use achieve a margin of exposure of 1000, the Agency believes that individuals are unlikely to experience adverse health effects from the termiticide use of chlorpyrifos. This conclusion is based on: the public health protective assumptions; the 1000 fold safety factor; and the additional 3 to 10 fold cushion between the NOAEL and the LOAEL. Mitigation measures will further reduce exposures and risk associated with the termiticide use. For example, the removal of whole house barrier treatment addressed the exposures of most concern. It is expected that the limited spot and localized treatment, and pre-construction treatments would represent less exposure and risk. In conclusion, based on the mitigation plan, and best professional and scientific judgement, the Agency concludes that the chronic aggregate risk including termiticide use, does not raise a concern.

Because of its extensive use, the majority of the U.S. population is exposed to chlorpyrifos or its environmental breakdown product, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). Epidemiology data have reported measurable concentrations of 3,5,6-TCP, which is also the primary metabolite of chlorpyrifos, chlorpyrifos-methyl and trichlorpyr in the urine of individuals. These data represent potential aggregate exposure to chlorpyrifos and/or 3,5,6-TCP from all exposure routes. 3,5,6-TCP was detected in the urine of 82% of 993 adults from the National Health and Nutrition Examination Survey III conducted between 1988 and 1993 (NHANES III). Preliminary results from the recent Minnesota Children's Exposure Study found that 92% of the 89 children evaluated had measurable urinary concentrations of 3,5,6-TCP. A 1998 biomonitoring study of 416 children in North and South Carolina found 3,5,6-TCP in urine of 100% of the children evaluated. TCP was found at higher average levels than all previous epidemiological studies of the general population. HED believes that chlorpyrifos contributes significantly more to urinary TCP than chlorpyrifos-methyl and trichlorpyr based on relative usage of 21-24 million pounds chlorpyrifos versus 92,000 pounds chlorpyrifos-methyl, and 700,000 pounds for trichlorpyr. Because chlorpyrifos, chlorpyrifos-methyl and trichlorpyr degrade to 3,5,6-TCP in the environment, exposure to TCP per se also contributes to the urinary 3,5,6-TCP residues to an unknown degree. As noted previously, HED conducted a screening-level risk assessment for TCP. This assessment is attached in memorandum from S. Knizner to D. Smegal, D265035 June 5, 2000.

2.0 Physical/Chemical Properties Characterization

Technical chlorpyrifos is a white crystalline solid with a melting point of 41.5-42.5° C. Chlorpyrifos is stable in neutral and acidic aqueous solutions; however, stability decreases with increasing pH. Chlorpyrifos is practically insoluble in water, but is soluble in most organic solvents (i.e., acetone, xylene and methylene chloride). Chlorpyrifos is not particularly volatile based on its low vapor pressure of 1.87×10^{-5} mmHg at 20°C (Merck Index, 11th Edition). Its maximum attainable vapor concentration is 25 ppb at 25° C.



Empirical Formula:	$C_9H_{11}Cl_3NO_3PS$
Molecular Weight:	350.6
CAS Registry No.:	2921-88-2
Chemical No.:	059101

The persistence of chlorpyrifos in soil varies depending on soil type, and environmental conditions. The typical aerobic soil metabolism half life ($T_{½}$) ranges from 11 to 180 days, with a mean of 28.7 days. Much longer soil half lives of 175 to 1576 days have been reported for termiticide application rates (Memorandum from M. Barrett to S. Knizner, Drinking Water Assessment of Chlorpyrifos, November 13, 1998, and memorandum from H. Nelson to D. Smegal/M. Hartman, October 6, 1999). The soil/water partition coefficient (K_{oc}) value ranges from 360 to 31000, indicating that it is not very mobile in soils.

Technical Grade Active Ingredient (TGAI) data requirements concerning the DAS 99% T (EPA Reg. No. 62719-44) and the 97% T (EPA Reg. No. 62719-15) are satisfied. Guideline 830.6314 (oxidatioin/reduction) data requirements remain outstanding for the DAS 99% T. There are 45 chlorpyrifos Manufacturing-Use Products (MPs). Data remain outstanding for many MPs. Product chemistry data requirements will be complete, provided that the registrants submit the data required as identified in the Revised Product and Residue Chemistry Chapter (Memorandum from S. Knizer to M. Hartman, October 1, 1999, D259613) for the chlorpyrifos MPs. In addition, the registrants must either certify that the suppliers of starting materials and the manufacturing processes for the chlorpyrifos technicals and manufacturing-use products have not changed since the last comprehensive product chemistry review or submit complete updated product chemistry data packages.

3.0 Hazard Characterization

3.1 Hazard Profile

The toxicological database is complete and adequate to support reregistration. in accordance with the Subdivision F Guidelines for a food use chemical.

Chlorpyrifos is moderately toxic following acute oral, dermal and inhalation exposures and is classified in toxicity category II for all exposure routes. Chlorpyrifos affects the nervous system by reversibly inhibiting the activity of cholinesterase (ChE), an enzyme necessary for the proper functioning of the nervous system. Inhibition of ChE is the most sensitive effect in all animal species evaluated and in humans, regardless of exposure duration. In animals, significant inhibition of plasma and red blood cell (RBC) ChE occur at doses below those that cause brain ChE inhibition. In animals, significant plasma and RBC ChE have been observed at oral doses as low as 0.025 to 0.3 mg/kg/day following exposure for two weeks to two years, while significant brain ChE inhibition has been observed at oral doses as low as 1 mg/kg/day following exposure for two weeks in pregnant rats (Hoberman 1998a,b). Female rats and especially pregnant rats appear to be more sensitive than adult male rats to cholinesterase inhibition (Moser et al. 1998, Hoberman 1998a,b, Mattsson et al. 1998). Data from two human studies suggest that humans (adult males) are similarly sensitive and possibly more sensitive than rats and dogs following acute and short-term oral exposure and acute dermal exposure based on plasma ChE inhibition and/or possible clinical signs. It is likely that the human sensitivity for ChE inhibition relative to rats (but not dogs) is due to species differences in the constituents of plasma ChE between rats and humans. For example, in rats, plasma ChE consists of approximately a 60:40 ratio of acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE), while in most humans and dogs, plasma ChE is predominately as BuChE, which is more sensitive to inhibition than AChE.

3.1.1 TCP

HED has concluded that the primary metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP), does not induce cholinesterase inhibition, and therefore is less toxic than chlorpyrifos (58 FR 19354, April 14, 1993). However, because of the potential exposure to TCP in food and residential settings, and evidence of increased susceptibility of rabbit fetuses relative to dams, HED conducted a screening-level risk assessment for TCP. This assessment is attached in a memorandum from S. Knizner to D. Smegal, D265035 June 5, 2000.

3.1.2 Neurotoxicity

Adult male rats acutely exposed to chlorpyrifos exhibited peak plasma ChE inhibition of 28-40% 3-6 hours after exposure at 1 mg/kg (Mendrala and Brzak 1998), while significant 30% RBC ChE inhibition was noted 4 hours following a single oral dose of 1.5 mg/kg (Zheng et al. 2000). Plasma, RBC and heart ChE inhibition of 45%, 17% and 19%, respectively were observed in female rats 24 hours following a single dose of 5 mg/kg (Dittenber 1997). The acute oral NOAEL for plasma ChE inhibition in male rats is 0.5 mg/kg/day. Clinical signs of neurotoxicity, in the absence of neuropathology, were observed in rats exposed to a single oral dose of 50 mg/kg as evidence by decreased motor activity, and increased incidence of clinical signs consistent with organophosphate intoxication. Chlorpyrifos was negative in the delayed neurotoxicity study in hens at single doses of 50, 100 or 110 mg/kg. Acute oral exposure to hens at 60 to 150 mg/kg caused 59-87% inhibition of neurotoxic esterase (NTE) 4-6 days after exposure (Capodicasa et al. 1991). In addition, delayed neuropathy was noted at 60-90 mg/kg which corresponded to 4-6 times the LD_{50} and required aggressive antidotal treatment. In rats, chlorpyrifos failed to inhibit NTE at single doses up to 100 mg/kg. There is evidence that NTE inhibition is related to organophosphate-induced delayed neuropathy (OPIDN).

Following longer-term exposures, there was no evidence of neurotoxicity or neuropathology in rats exposed at doses up to 15 mg/kg/day for 13 weeks. However, in the developmental neurotoxicity study, pregnant dams exposed to chlorpyrifos for approximately 2 weeks exhibited 43% and 41% inhibition of plasma and RBC ChE activity at 0.3 mg/kg/day, significant 18% brain ChE inhibition at 1 mg/kg/day, and clinical signs of neurotoxicity, including fasciculations (muscle twitching), hyperpnea (increased respiration), and hyperactivity in addition to decreased body weight gain at 5 mg/kg/day (Hoberman 1998a,b). Cholinesterase inhibition (68% plasma, 56% RBC and 8% brain) was also noted in rats exposed to 1 mg/kg/day chlorpyrifos for 4 weeks in the cognitive study, while clinical signs of toxicity were not observed until higher doses of 3 mg/kg/day for miosis (pupil contraction) and 10 mg/kg/day for salivation and tremors (Maurissen et al. 1996).

3.1.3 Subchronic Toxicity

Several subchronic studies are available for chlorpyrifos including two oral rat studies, one oral dog study, a 21 day dermal toxicity study in rats, and two inhalation studies in rats. The most sensitive effect following subchronic oral exposure is inhibition of plasma ChE in rats and dogs at 0.025 to 0.03 mg/kg/day, and RBC ChE inhibition in dogs and rats at 0.22 to 0.3 mg/kg/day. Rats exposed to higher doses exhibited hematological

effects at doses of 10 mg/kg/day and increased brain and heart weight, adrenal gland effects and decreased body weight gain at 15 mg/kg/day. No adverse effects were noted in rats exposed via inhalation to the highest attainable vapor concentration of 20.6 ppb (287 Fg/m^3) (0.1 mg/kg/day). No adverse effects were observed in the 21-day dermal study in rats at doses as high as 5 mg/kg/day. However, in a 4-day dermal probe study, rats dermally exposed to doses of 0, 1, 10, 100, or 500 mg/kg/day exhibited reductions in plasma and RBC ChE activities at doses of 10 to 500 mg/kg/day. The 21-day dermal NOAEL is 5 mg/kg/day based on a 45% and 16% inhibition of plasma and red blood cell cholinesterase, respectively in rats dermally exposed to 10 mg/kg/day for 4 days.

3.1.4 Carcinogenicity/Genotoxicity

Chlorpyrifos was evaluated for carcinogenic potential in both rats (2 studies), and mice (2 studies). There was no evidence of carcinogenicity. Chlorpyrifos is not mutagenic in bacteria, or mammalian cells, but did cause slight genetic alterations in yeast and DNA damage to bacteria. In addition, chlorpyrifos did not induce chromosome aberrations in vitro, was not clastogenic in the mouse micronucleus test in vivo, and failed to induce unscheduled DNA synthesis in isolated rat hepatocytes.

3.1.5 Chronic Toxicity

Chlorpyrifos was evaluated for chronic toxicity in rats, mice and dogs. In all animal species, the most sensitive effect is inhibition of plasma, RBC and brain ChE that occurred at levels in the range of 0.03 to 3 mg/kg/day. Following chronic exposure dogs appear to be the most sensitive species for cholinesterase inhibition and systemic effects, as noted by increased liver weights in dogs exposed to 3 mg/kg/day that could be an adaptive response. Rats exposed to 7-10 mg/kg/day had decreased body weight and decreased body weight gain, ocular effects, adrenal gland effects and altered clinical chemistry and hematological parameters. Mice appear to be the least sensitive to chronic oral doses of chlorpyrifos, as exposure to 45-48 mg/kg/day resulted in decreased body weight and an increased incidence of non-neoplastic lesions (i.e., keratitis, hepatocyte fatty vacuolation).

3.1.6 Developmental Toxicity

Chlorpyrifos was evaluated for developmental toxicity in rats, mice and rabbits. In one rat study, developmental effects (increased post-implantation loss) were noted at 15 mg/kg/day (highest dose tested, HDT), that were also associated with maternal toxicity, while another rat study failed to observe developmental effects at 15 mg/kg/day. Developmental effects were also noted at higher doses in mice at 25 mg/kg/day (minor skeletal

variations, delayed ossification and reduced fetal weight and length) and rabbits at 140 mg/kg/day (decreased fetal weights and crown rump lengths, and unossified xiphisternum and/or 5th sternebra). However, in both mice and rabbits, the developmental effects occurred at maternally toxic doses as indicated by reduced weight gain, and food consumption in both species, and increased mortality in mouse dams.

In the rat developmental neurotoxicity study, chlorpyrifos was associated with delayed alterations in brain development in offspring of exposed mothers. Specifically, pups of the 1 mg/kg/day group exhibited significant dose- and treatment-related decreases in measurements of the parietal cortex in female offspring at postnatal day 66. The only maternal effect at this dose was plasma and RBC ChE inhibition. At higher doses, pups of the 5 mg/kg/day group exhibited decreased body weight/body weight gain and food consumption in both sexes, reductions in pup viability, delays in development, decreased brain weight and morphometric alterations in the brain. However, these effects were observed in the presence of maternal toxicity as evidenced by fasciculations, hyperpnea and hyperactivity, in addition to reduced body weight gain.

Several studies in the peer reviewed literature and results of the guideline developmental neurotoxicity study are supportive of the possibility that chlorpyrifos exposure may affect brain development (e.g., altered synaptic development, alterations in DNA, RNA, and protein synthesis, inhibition of mitosis and mitotic figures, and disruption of the structural architecture of the brain) (Whitney et al. 1995, Campbell et al. 1997, Song et al. 1997, Johnson et al. 1998, Das and Barone 1999, Dam 1999, Roy et al. 1998, Hoberman 1998a,b). There are suggestive data that these effects may arise independent of cholinesterase inhibition.

3.1.7 Reproductive Toxicity

Chlorpyrifos induced reproductive toxicity in one generation of rats, but only at dose levels that induced parental toxicity. Reproductive effects included reduced pup weights and increased pup mortality that corresponded to slightly but significantly reduced body weight gain in F0 dams during lactation days 1-21, in addition to parental toxicity as evidenced by inhibition of plasma, RBC and brain cholinesterase activities as well as histological lesions of the adrenal gland (vacuolation of cells of the zona fasciculata).

3.1.8 Human Studies

HED has reviewed two human studies conducted with chlorpyrifos submitted by the registrant (MRID 95175, Accession No. 249203). A third

human study (Kisicki et al. 1999) that evaluated a single dose exposure was submitted on April 27, 1999 but is an incomplete submission because two Appendices with critical data were omitted. In the first study (MRID No. 95175; Coulston et al., 1972), male volunteers from Clinton Correctional Facility (4/dose group) were given daily oral (tablet) doses of 0, 0.014, 0.03, or 0.1 mg/kg chlorpyrifos technical for 7 weeks, 9 days, 21 days and 28 days, respectively. Significant 36-82% plasma ChE inhibition relative to baseline was observed after 9 days of treatment with 0.1 mg/kg/day chlorpyrifos. In addition, one of the four men in the 0.1 mg/kg/day developed blurred vision, runny nose and a feeling of faintness on day 9. Exposure was discontinued on day 9 in this dose group however, due to plasma cholinesterase inhibition that exceeded the study investigator's guideline of 20%-30%. No significant plasma ChE inhibition was observed in the men exposed to 0.03 mg/kg/day for 21 days or at any other dose that could be attributed to treatment. No effects on RBC ChE were found at any dose that could be attributed to treatment. A gradual recovery was observed in plasma ChE values equaling baseline values by day 25 of the recovery period. The registrant and study director contend that the clinical signs were attributed to a cold, and not chlorpyrifos exposure. HED believes that blurred vision is a typical cholinergic sign of ChE inhibition, and can not be attributed to a common cold (February 2, 1998 HIARC Report, HED Doc No. 012471). In addition, there is no reason to believe that other clinical signs would not have appeared if the dosing had continued for 21 or 28 days as it did for the other groups. While the study director claims that exposure to the high dose group was discontinued on day 9 because plasma ChE inhibition was 20-30%, rather than because of concern for the clinical signs, this reason is inconsistent with the study findings of 46% mean plasma ChE inhibition following day 6 of treatment in the 0.1 mg/kg/day group, and 41% plasma ChE inhibition in one individual on day 3. HED notes that the relatively long recovery period of 25 days is unusual for plasma ChE, and is more characteristic of recovery for RBC acetyl ChE inhibition based on the 2 year dog data (McCollister et al. 1971, Kociba et al. 1985).

An acute oral and dermal pharmacokinetic study (Nolan et al. 1982, Accession No. 249203) dosed six men once with 0.5 mg/kg orally and four weeks later dosed five of these same men with 5 mg/kg dermally, and one man with 0.5 mg/kg dermally. No clinical signs or symptoms were observed in any of the subjects, but unlike the previous study, the primary focus of this study was pharmacokinetics. Men orally exposed to 0.5 mg/kg chlorpyrifos exhibited peak plasma ChE inhibition of 64-85%, 12 to 24 hours post-exposure. Peak RBC ChE inhibition of 11-52% occurred on post-exposure day 4. Men dermally exposed to 5 mg/kg chlorpyrifos exhibited peak plasma ChE inhibition of 27-45% on day 3, and mean RBC ChE inhibition of 8.6% on day 4. The return of plasma ChE activity to pre-dose levels required about 30 days. The registrant stated that the inhibition noted on days 3 and 4 is an analytical artifact based on chlorpyrifos

pharmacokinetics. If this is the case, it raises concerns about the quality and reliability of the study data. Again, HED notes that the relatively long recovery period of 30 days is unusual for plasma ChE, and is more characteristic of recovery for RBC acetyl ChE inhibition based on the 2 year dog data (McCollister et al. 1971, Kociba et al. 1985). On the basis of urinary excretion of the 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) metabolite, the minimum oral absorption of chlorpyrifos was estimated at 70% and the minimal dermal absorption at 1-3%. Because the proportion of the administered dose metabolized to this pyridinol is unknown, these estimates are considered minimum values (i.e., absorption could be higher). The mean pharmacokinetic half-life for 3,5,6-TCP in the urine was approximately 27 hours following both oral and dermal exposure.

As noted previously, data from the two human studies suggest that humans are as sensitive and possibly more sensitive than animals based on plasma ChE inhibition and possible clinical signs. For example, in animals (rats), the acute oral (single dose) NOAEL is 0.5 mg/kg/day, while humans exposed to a single oral 0.5 mg/kg/day dose exhibited 64-85% plasma ChE inhibition. Based on an overall assessment of the plasma and RBC ChE inhibition data, the HIARC identified an animal NOAEL and LOAEL of 0.03 mg/kg/day and 0.22-0.3 mg/kg/day, respectively for longer term exposures (several months), while humans exposed to 0.1 mg/kg/day for only 9 days exhibited 36-82% plasma ChE inhibition and possible clinical signs (blurred vision). The short-term dermal NOAEL in rats is 5 mg/kg/day based on plasma and RBC ChE inhibition observed at 10 mg/kg/day, while humans exposed dermally for one day to 5 mg/kg/day exhibited 27-45% plasma ChE inhibition. For all endpoints based on rat data, it is likely that this sensitivity can be attributed to species differences in plasma ChE between the rat and humans. For example, in rats, plasma ChE consists of approximately a 60:40 ratio of acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE), while in most humans and dogs, plasma ChE is predominately as BuChE, which is more sensitive to inhibition than AChE.

3.1.9 Metabolism/Pharmacokinetic Studies.

In the rat, chlorpyrifos is excreted primarily in the urine (84%) with lesser amounts excreted in the feces (5%) within 72 hours. The metabolism of chlorpyrifos was extensive, and no unchanged parent compound was found in the urine. The major urinary metabolites were 3,5,6-TCP, as well as glucuronide and sulfate conjugates of TCP.

As noted previously, in humans (adult males) approximately 70% of chlorpyrifos is excreted in the urine as TCP within 5 days following acute oral exposure, and the minimum dermal absorption is 1 to 3% (Nolan et al. 1982, Accession No. 249203). The mean pharmacokinetic half-life for 3,5,6-TCP in the urine was approximately 27 hours following both oral and dermal

exposure.

3.1.10 Sensitivity/Susceptibility of the Young

A number of studies published in the scientific literature have also been considered by the Agency and are discussed in the Hazard Identification and Assessment Review Committee (HIARC) April 6, 2000 report (HED No. 014088), February 2, 1998 report (HED No. 012471) and December 7, 1998 report (HED No. 013004). Summaries of several of these studies are presented in the attached Toxicology Chapter memorandum from D. Smegal to M. Hartman, April 18, 2000, D263892, and in the report "Chlorpyrifos Children's Hazard: Sensitivity and Susceptibility" March 28, 2000, HED No. 014074 (which is an appendix to the April 6, 2000 HIARC report). The HIARC concluded that there is sufficient evidence in the scientific literature to suggest that exposure to chlorpyrifos results in increased sensitivity and susceptibility to neonates as compared to adult rats. The Weight of Evidence Characterization and Conclusions of the "Chlorpyrifos Children's Hazard: Sensitivity and Susceptibility" document (March 28, 2000, HED No. 014074) are presented in Appendix A.

3.1.11 Paraoxonase

Chlorpyrifos, and some other organophosphate (OP) compounds, are detoxified via a two-step pathway involving bioactivation of the parent compound to an oxon by the cytochrome P450 systems, and then hydrolysis of the resulting oxon compounds by esterases such as liver or serum paraoxonase (PON1) (located in the plasma) (Davies et al. 1996, Furlong et al. 1998, Shih et al. 1998). In the human population, serum PON1 activity is genetically determined (polymorphic) and individuals express widely different levels of this enzyme (Davies et al. 1996). Therefore, it is possible that some individuals may be more sensitive to chlorpyrifos toxicity based on genetic factors that regulate serum PON1 activity resulting in a reduced capacity to detoxify chlorpyrifos-oxon. Paraoxonase data were collected for individuals in a recent single dose human study (Kisicki et al. 1999). HED will evaluate these data once they are submitted to the Agency.

In animals, there is evidence that serum paraoxonase is protective against poisoning by OPs. Animals with low PON1 levels were more sensitive to specific OP compounds than animals with high enzyme levels. For example, birds, which have very low to undetectable PON1 activity are more sensitive than various mammals to the acute toxicity of oxons for other OPs (paraoxon, diazinon oxon and pirimiphos oxon). Further rabbits, which have a sevenfold higher serum PON1 activity than rats, are more resistant to the acute toxicity of chlorpyrifos (approximately 9 and 25 fold for acute oral and dermal toxicity, respectively). Rabbit paraoxonase hydrolyzes chlorpyrifos-oxon with a much higher turnover number than does rat paraoxonase (Costa et al. 1999, Li et al. 1993).

3.2 Acute Toxicity

Chlorpyrifos is moderately toxic following acute oral, dermal and inhalation exposures, and is classified in toxicity category II for all three routes of exposure for rats. The oral LD_{50} values for technical chlorpyrifos are higher in rats (223 mg/kg) than mice (62.5 mg/kg, toxicity category II) or chicks (32 mg/kg, toxicity category 1). Female rats are more sensitive (i.e., lower LD_{50}) than male rats for both technical chlorpyrifos and formulated products. Guinea pigs and rabbits are less sensitive to acute toxicity than rats as noted by the oral LD_{50} values of 504 mg/kg and 1000-2000 mg/kg, respectively (both category III), and the rabbit dermal LD_{50} value of >5000 mg/kg (category IV). Chlorpyrifos was not acutely neurotoxic when given to hens at a single oral dose of 50 mg/kg (the LD_{50}), 100 or 110 mg/kg. In rats, the LC_{50} was greater than 0.2 mg/L (or 200 mg/m^3), which is normally assigned toxicity category II. This study is classified as Supplementary because only nominal concentrations were measured. Acute toxicity values and categories for the technical grade of chlorpyrifos are summarized in the following table.

Table 1. Acute Toxicity Results for Technical Chlorpyrifos

STUDY	MRID Number	RESULTS	CATEGORY
Acute Oral LD_{50} - rat	44209101	223 mg/kg M&F	II
Acute Dermal LD_{50} - rat	Accession No. 112115	202 mg/kg	II
Acute Dermal LD_{50} - rabbit	44209102	>5000 mg/kg	IV
Acute Inhalation LC_{50}; rat Supplementary	00146507 and Accession No. 257590	LC_{50} > 0.2 mg/L (200 mg/m^3) (nominal concentration)	II
Eye Irritation - rabbit	44209103	slight irritation resolved within 24 hours	IV
Dermal Irritation - rabbit	44209104	mild irritant; (irritation resolved within 7 days)	IV
Dermal Sensitization - guinea pig	44209105	non-sensitizing	NA
Acute Delayed Neurotoxicity in hens	00097144 00405106	not neurotoxic at 50, 100 or 110 mg/kg	NA

NA = not applicable

3.3 FQPA Considerations

In March 1999, the FQPA Safety Factor Committee (SFC) recommended that an FQPA safety factor was needed due to concern for increased sensitivity seen at high doses in a literature study comparing adults and neonates, and for the qualitative increased susceptibility occurring at the high dose in the developmental neurotoxicity study. Nonetheless, the FQPA safety factor was reduced to 3X because of lack of data addressing whether or not these differences would also occur at lower doses. A re-evaluation of this recommendation was conducted by the FQPA SFC on January 24, 2000. The new evaluation was undertaken in order to consider the possible impact of new hazard information received in the last year (Slotkin 1999, Zheng et al. 2000). At the January 24th meeting, however, the Committee members were unable to reach consensus on the safety factor recommendation. Subsequently, arguments for retention of the safety factor at 10X or reduction of the safety factor to 3X were presented, with supporting information for review, to the OPP Division Directors and several Agency senior scientists at a February 7, 2000 meeting. The Division Directors and senior scientists (DD-SS group), recommended that the FQPA safety factor should be **retained at 10X** for the protection of infants and children to exposure resulting from chlorpyrifos. The details of this decision are presented in the attached memo from B. Tarplee 4/4/00 HED Doc No. 014077. The DD-SS group recommended that a 10X safety factor be retained for chlorpyrifos due to:

In February 2000, new data (Zheng et al. 2000, Hoberman 1998a,b) demonstrated that the increased sensitivity and susceptibility was not only a high dose phenomenon since:

< increased sensitivity following a single oral exposure to neonates was seen at substantially lower doses (Zheng et al. 2000, in press); and

< a clear qualitative difference in response (i.e., susceptibility) between adult rats and their offspring was demonstrated in the developmental neurotoxicity (DNT) study (cholinesterase inhibition in dams versus structural effects on developing brain of the offspring) (Hoberman 1998a,b).

New data in the literature also gave rise to uncertainties such as:

< the suggestion that the inhibition of cholinesterase may not be essential for adverse effects on brain development; and

< the lack of an offspring NOAEL in the DNT based upon structural alterations in brain development as the toxicity endpoint of concern.

Therefore, the DD-SS group concluded that their evaluation of the available hazard and exposure databases for chlorpyrifos, including the information received and

reviewed in the past year, results in an overall ***higher*** degree of concern regarding the potential consequences of chlorpyrifos exposure to infants and children than was determined during the FQPA safety factor evaluation in March 1999. Consequently, they recommended that the FQPA safety factor should be Retained at 10X for the protection of infants and children to exposure resulting from the use of chlorpyrifos.

The FQPA SFC determined that the FQPA safety factor would be applicable to **Females 13-50** and **Infants and Children** population subgroups for **all exposure durations**:

Acute Dietary Assessment - The FQPA safety factor is applicable for Females 13-50 and Infants and Children population subgroups due to the concern that adverse effects could result from a single exposure to chlorpyrifos (as demonstrated in several open literature studies including Zheng et al.).

Chronic Dietary Assessment - The FQPA safety factor is applicable for Females 13-50 and Infants and Children population subgroups due to the concern that potential adverse effects could result from repeated exposure to chlorpyrifos (as demonstrated, for example, in the developmental neurotoxicity study in rats).

Residential and other Non-occupational Exposure Assessment - The FQPA safety factor is applicable for Females 13-50 and the Infants and Children population subgroups for all exposure durations due to the adverse effects resulting from single or repeated exposure(s) to this organophosphate insecticide in or around residential (non-occupational) settings.

3.4 Endpoint Selection

It is current Agency policy that a regulatory decision can not be made based on a human study until a formal decision has been made concerning the ethical aspects of such use. The ethics decision regarding the use of toxicology studies employing human subjects has not yet been made. Therefore, the Agency selected doses and endpoints to calculate dietary and non-dietary risk in the current assessment based solely on animal studies.

There are three human studies available for chlorpyrifos, however one of these studies is an incomplete submission (Kisicki et al. 1999). The HED HIARC met on January 5, 1999 to evaluate the scientific quality of the two human studies which were the basis of the previous RfDs and dermal and inhalation risk assessment endpoints. This re-evaluation was initiated because of a joint Science Advisory Panel/Science Advisory Board (SAP/SAB) meeting held in December 1998 that discussed issues surrounding the scientific and ethical concerns for human toxicity testing. The HIARC committee concluded that both human studies (Coulston et al. 1972 MRID No. 00095175, Nolan et al. 1982, MRID No. 00249203)

provided useful scientific information that can be used as supportive data along with the results of animal studies. However, these studies alone are not sufficient for endpoint selection or use in risk assessment primarily because of the small sample size (n=4-6/dose group), evaluation of only adult males (when females tend to be more sensitive), insufficient information on study protocol, and lack of control for confounding factors. In addition, the Nolan et al. (1982) pharmacokinetic study only tested one dose level. Furthermore, the registrant contends that the plasma and RBC ChE activity data results on day 3 and 4 of the Nolan et al. (1982) study are analytical artifacts, which raises concerns about the quality and reliability of the study data.

The HIARC met on February 2, 1999 and re-assessed the toxicology database to select toxicology endpoints based on animal studies for dietary and non-dietary exposure risk assessments. On January 20, 2000, and March 28, 2000 the Committee re-convened to address issues raised during the Phase 3 public comment period. The Committees decisions are presented in the attached HIARC memorandum dated April 6, 2000 (D. Smegal to S. Knizner, HED Doc No. 014088). The doses and toxicological endpoints selected for various exposure scenarios based on animal toxicity studies with chlorpyrifos are summarized in Table 2.

Table 2
Summary of Doses and Endpoints Selected for Chlorpyrifos Risk Assessment

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	Target MOE for Workers	Target MOE for Non-Occupational
Acute Dietary	NOAEL=0.5 UF = 100 FQPA = 10 (infants,children and females 13-50)	Significant (28-40%) plasma cholinesterase inhibition at peak time of inhibition (3-6 hours post exposure) at 1 mg/kg (Mendrala and Brzak 1998). Significant 30% RBC ChE inhibition 4 hours post exposure to 1.5 mg/kg/day (Zheng et al. 2000).	Acute Blood Time Course Study in male rats (Mendrala and Brzak 1998) with support from Zheng et al. (2000)	NR	NR
	Acute RfD =0.005 mg/kg/day **Acute PAD (children and females 13-50) = 0.0005 or $5x10^{-4}$ mg/kg/day** **Acute PAD (general population) = 0.005 or $5x10^{-3}$ mg/kg/day**				
Chronic Dietary	NOAEL= 0.03 UF= 100 FQPA = 10 (infants,children and females 13-50)	Significant plasma and RBC cholinesterase inhibition at 0.22 to 0.3 mg/kg/day	Weight of Evidence from 5 studies: 2 year dog 90 day dog 2 year rat 90 day rat developmental neurotoxicity (DNT) study (at 2 weeks)	NR	NR
	Chronic RfD =0.0003 mg/kg/day **Chronic PAD (children and females 13-50) = 0.00003 or $3x10^{-5}$ mg/kg/day** **Chronic PAD (general population) = 0.0003 or $3x10^{-4}$ mg/kg/day**				
Short-Term (Dermal)	Dermal NOAEL =5 Absorbed Dermal NOAEL = 0.15 (for biomonitoring) (a)	Plasma and RBC cholinesterase inhibition of 45 and 16%, respectively at 10 mg/kg/day after 4 days. (Dermal absorption factor not necessary for administered dermal NOAEL)	21-day dermal rat study	100	1000 (infants,children and females 13-50) 100 (males)

Table 2
Summary of Doses and Endpoints Selected for Chlorpyrifos Risk Assessment

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	Target MOE for Workers	Target MOE for Non-Occupational
Intermediate- and Long-Term (Dermal)	Oral NOAEL =0.03 (3% dermal absorption)	Significant plasma and RBC cholinesterase inhibition at 0.22 to 0.3 mg/kg/day	Weight of Evidence from 5 studies: 2 year dog 90 day dog 2 year rat 90 day rat DNT study (at 2 weeks)	100	1000 (infants,children and females 13-50) 100 (males)
Short-,and Intermediate-Term (Inhalation)	Inhalation NOAEL= 0.1	Lack of effects in 2 rat inhalation studies at the highest dose tested; 43% plasma and 41% RBC cholinesterase inhibition following oral doses of 0.3 mg/kg/day for 2 weeks in the DNT study	Two 90 day rat inhalation studies (NOAEL) and DNT (LOAEL)	100	1000 (infants,children and females 13-50) 100 (males)
Long-Term (Inhalation)	Oral NOAEL= 0.03 (assume inhalation absorption is 100% of oral absorption)	Significant plasma and RBC cholinesterase inhibition at 0.22 to 0.3 mg/kg/day	Weight of Evidence from 5 studies: 2 year dog 90 day dog 2 year rat 90 day rat DNT (at 2 weeks)	100	1000 (infants,children and females 13-50) 100 (males)

RBC = red blood cell
NR = not relevant
UF = Uncertainty Factor
MOE = Margin of Exposure
PAD = Population Adjusted Dose (includes UF and FQPA safety factor)
(a) Use absorbed dermal NOAEL of 0.15 mg/kg/day (5 mg/kg/day * 0.03 dermal absorption factor) for comparison with absorbed biomonitoring exposure.

3.5 Endocrine Disrupter Effects

The Food Quality Protection Act (FQPA; 1996) requires that EPA develop a screening program to determine whether certain substances (including all pesticides and inerts) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect...." EPA has been working with interested stakeholders, including other government agencies, public interest groups, industry and research scientists to develop a screening and testing program as well as a priority setting scheme to implement this program. The Agency's proposed Endocrine Disrupter Screening Program was published in the Federal Register of December 28, 1998 (63 FR71541). The Program uses a tiered approach and anticipates issuing a Priority List of chemicals and mixtures for Tier 1 screening in the year 2000. As the Agency proceeds with implementation of this program, further testing of chlorpyrifos and its end-use products for endocrine effects may be required.

4.0 Exposure Assessment

4.1 Summary of Registered Uses

Chlorpyrifos is a broad-spectrum, organophosphate insecticide that was first registered in 1965 to control foliage- and soil-borne insect pests on a variety of food and feed crops. It is one of the most widely used organophosphate insecticides in the U.S. and is one of the major insecticides used in residential settings. There are approximately 822 registered products containing chlorpyrifos on the market (REFs 9/14/99). Registered uses include: a wide variety of food crops (i.e., there are approximately 112 tolerances for food and/or feed commodities such as citrus, vegetable crops, tree fruits, etc); turf and ornamental plants; greenhouses; sodfarms; indoor pest control products (e.g., crack and crevice); structural pest control (e.g., termites); and in pet collars. Indoor uses include residential and commercial buildings, schools, daycare centers, hotels, restaurants and other food handling establishments, hospitals, stores, warehouses, food manufacturing plants, vehicles, livestock premises, and mushroom houses. In addition, it is used as an adult mosquitocide and is registered for ear tag treatment of cattle (beef and lactating and non-lactating dairy). Chlorpyrifos products are widely used by both homeowners and LCOs/PCOs.

BEAD estimates that the annual total domestic usage of chlorpyrifos is approximately 21 to 24 million pounds ai for 8 million acres treated in the U.S. Approximately 11 million pounds are applied annually in non-agricultural settings (i.e., residences, schools, golf courses, parks). Chlorpyrifos has the largest agricultural market in terms of total pounds ai allocated to corn (26%). The largest non-agricultural markets in terms of total pounds ai applied are PCOs, termite control (24%), and turf (12%). Crops with a high average percentage of their total

U.S. planted acres treated include brussel sprouts (73%), cranberries (46%), apples (44%), broccoli (41%) and cauliflower (31%).

Comprehensive lists of chlorpyrifos end-use products (EPs) and of use patterns with food/feed uses which are subject to re-registration appear are summarized in the Revised Product and Residue Chapter (Memorandum from S. Knizner to M. Hartman, June 2000).

The formulations registered for use on food and feed crops include the granular (G), wettable powder (WP), impregnated material (Impr), dry flowable (DF), and emulsifiable concentrate (EC). Dry flowable and wettable powder in open bags are not assessed and no longer are eligible for re-registration. These formulations may be applied as foliar, bark, seed, and soil-incorporated band or broadcast treatments using ground, sprinkler irrigation, or aerial equipment. The different crop growth stages or timings as to when chlorpyrifos formulations may be applied are dormant, delayed dormant, preplant, at-planting, transplanting, postplant, post-transplant, preemergence, and postemergence. The impregnated material formulation is registered for ear tag use on cattle. The chlorpyrifos formulations registered for food-handling establishments include the microencapsulated (Mcap), emulsifiable concentrate, and liquid ready-to-use (RTU) and soluble concentrate (SC/L) [Source: REFS 9/99].

4.2 Dietary Exposure

OPP has determined that TCP is not of toxicological concern and can be excluded from the tolerance expression because it does not inhibit cholinesterase (PP3F2884 and 3F2947 and FAP3H5396 and 3H5411/R1191, Final Rule, D.Barolo, 4/1/93). The conclusions specified in the "Tolerance Reassessment Summary" section of the Revised Product and Residue Chemistry Chapter (Memorandum from S. Knizner to M. Hartman, June 2000) reflect this decision and recommendation to consider only chlorpyrifos *per se* as the residue of concern. HED conducted a screening-level TCP assessment (memorandum from S. Knizner to D. Smegal, June 5, 2000, D265035).

4.2.1 Residue Chemistry Data Requirements

Plant and Animal Metabolism. The qualitative nature of the residue in plants and animals is adequately understood based on acceptable metabolism studies with a cereal grain (corn), a root and tuber vegetable (sugar beets), and acceptable poultry and ruminant metabolism studies. The residue of concern in plants and animals is chlorpyrifos *per se*. There are presently no direct application uses of chlorpyrifos on meat- and milk-producing animals, except for ear tag treatment of cattle (beef and lactating and non-lactating dairy).

Residue Analytical Methods - Plants and Animals. The requirements for residue analytical methods are fulfilled for purposes of re-registration. In consideration of HED's decision to regulate only the parent chlorpyrifos, acceptable methods are available for enforcement and data collection purposes. The behavior of chlorpyrifos using FDA's multi residue protocols has also been investigated and reported.

Storage Stability. The requirements for storage stability data are fulfilled for purposes of reregistration. Acceptable storage stability studies have been conducted on representative oil seeds, non-oily grains, root crops, fruits and fruiting vegetables, and low moisture content forage and hay. Additional studies have also been conducted to investigate the frozen stability of chlorpyrifos in selected processed food/feed commodities and in animal tissues and milk.

Magnitude of the Residue. The reregistration requirements for magnitude of the residue in plants (crop field trials and processed food/feed commodities) are fulfilled for the majority of crops. There are minor data gaps for asparagus, corn, cotton, crops grown solely for seed (clover and grasses), mint, peppers, sorghum, tomatoes, tree nut group and wheat. The reregistration requirements for magnitude of the residue in food-handling establishments are fulfilled. Sufficient data exist to determine that when registered formulations are used according to label directions, no detectable residues (<0.01-<0.025 ppm) are likely to occur in food items. Bait and insecticidal strip uses would not result in residues greater than those resulting from spray applications. Therefore, the outstanding data are considered confirmatory.

The reregistration requirements for magnitude of the residue in animals are fulfilled. There are presently no registered direct application uses of chlorpyrifos on livestock animals except for ear tag treatment of cattle (beef and lactating and non-lactating dairy). An acceptable residue transfer study of chlorpyrifos to milk and cream from dairy cows wearing chlorpyrifos-impregnated tags has been submitted; data from this study indicate that residues in whole milk and fat resulting from ear tag use should not be a significant fraction of the residues resulting from intake of animal feeds containing chlorpyrifos. Cattle and poultry feeding studies have been evaluated and found adequate to satisfy feeding study requirements.

Confined/Field Rotational Crops. Provided that the Registrant modifies all labels for its chlorpyrifos containing products to limit application to 5 lb ai/A/season on those crops where rotation to another crop could occur (as was stated in their letter to the Agency dated 8/12/94), HED will not require field rotational crop studies. Furthermore, a 30 day plant back interval for rotational crops would then be appropriate.

4.3 Dietary Exposure (Food Source)

As noted previously, chlorpyrifos is registered for use on a wide variety of food crops, and has approximately 112 tolerances for food and/or feed commodities (which translates to approximately 700 food forms in the dietary analysis). Food uses evaluated in this analysis were those reflected by the established tolerances in/on raw agricultural, animal, and processed food/feed commodities for chlorpyrifos as listed in 40 CFR §180.342. Food handling establishment (FHE) tolerances were also included as cited in 40 CFR §185.1000 for the chronic dietary analysis (i.e., as a result of the registered use in FHE, all foods have an established tolerance of 0.1 ppm, unless they are covered by higher tolerances). The tolerances published for chlorpyrifos under 40 CFR §180.342, 185.1000 and 186.1000 have been reassessed (HED Revised Product and Residue Chemistry Chapter, memorandum from S. Knizner to M. Hartman, June 2000). The established tolerances in/on raw agricultural, animal, and processed food/feed commodities are expressed either in terms of the combined residues of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol (TCP) or as chlorpyrifos *per se.* HED has determined that TCP is not of toxicological concern and concluded that TCP can be excluded from the tolerance expression. Reassessed tolerances are in terms of chlorpyrifos *per se*. Thus, for purposes of this analysis, only residues of chlorpyrifos *per se* were considered, when data were available. Whenever possible, data for anticipated residues (ARs) reflect levels of chlorpyrifos *per se*. HED has conducted a screening-level risk assessment for TCP, which is in the attached memorandum from S. Knizner to D. Smegal, D265035 June 5, 2000.

Highly refined acute and chronic dietary exposure assessments were conducted using the Dietary Exposure and Evaluation Model (DEEM™) system. DEEM can be used to estimate exposure to residues in foods comprising the diets of the U.S. population, including population subgroups. The software contains food consumption data from the USDA Continuing Survey of Food Intake by Individuals (CFSII) from 1989-1992. For chronic dietary risk assessments, the 3-day average of the consumption data for each sub-population is combined with average residues in commodities to determine the average exposure in mg/kg/day. For acute dietary risk assessment, the entire distribution of single day food consumption events is combined with a distribution of residues (probabilistic analysis, referred to as "Monte Carlo") to obtain a distribution of exposures in mg/kg/day.

For chlorpyrifos, inputs to the DEEM analysis include DAS' National Food Survey (NFS, 1993 - 1994), U.S. Department of Agriculture's Pesticide Data Program (PDP) monitoring data (1994-1999), the Food and Drug Administration (FDA) Surveillance Monitoring Program data (1992-1998), and to a much lesser extent, field trial residue data. Percent crop treated data were supplied by the Biological and Economic Analysis Division (Quantitative Usage Analysis for Chlorpyrifos dated 3/30/00). Where percent crop treated estimates indicated no

chlorpyrifos use, a default minimum assumption of 1% crop treated was applied. In general, when residues on commodities were nondetectable, one-half the limit of detection (LOD) was assumed. All available processing and cooking factors were incorporated into the dietary exposure analysis.

At their own initiative, DAS conducted a market basket survey (NFS), with samples collected from the Fall of 1993 to the Fall of 1994, to better determine the dietary exposure of consumers to chlorpyrifos. The results of this survey have been reviewed by HED (L. Cheng, 5/19/98, D217707). Samples of fresh apple, applesauce, apple juice, orange juice, peanut butter, whole milk, ground beef and pork sausage were collected from grocery stores located in the 48 contiguous states; for fresh tomatoes, sampling was conducted in Florida only over a period of 9 months, because the domestic use of chlorpyrifos was restricted to Florida at the time of sampling. Approximately 200 samples were collected for each commodity, except for tomatoes, where 55 samples were collected. The nine food items were selected because of their significant contributions to dietary exposure in general (and in infants and children), and the potential for high residues based on modes of application and the percentage of crop treated. The apple and tomato samples were composite samples consisting of six apples and four tomatoes, respectively.

The Reference Dose (RfD) is derived from an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control, along with the application of uncertainty factors. The percent of the RfD is calculated as the ratio of the exposure value to the RfD (exposure/RfD x 100 = % RfD). The population adjusted dose (PAD) is the adjusted RfD reflecting the application of the FQPA safety factor. The FQPA safety factor for females and children is 10X, for all other populations subgroups it is 1X. For females and children, the population adjusted doses for acute and chronic dietary risk assessment are 0.0005 mg/kg/day and 0.00003 mg/kg/day, respectively. For all other population subgroups, the population adjusted doses for acute and chronic dietary risk assessment are 0.005 mg/kg/day and 0.0003 mg/kg/day, respectively. Exposures less than 100% of the PAD do not exceed HED's level of concern.

4.3.1 Acute Dietary Exposure Assessment

The HED probabilistic acute dietary exposure estimates used PDP, and FDA monitoring data to the greatest extent possible, in conjunction with the DAS's NFS data for all commodities included in the survey except apples and tomatoes. NFS data were used for milk, apple juice, applesauce, orange juice, ground beef, pork sausage, and peanut butter. A summary of the acute dietary analysis can be found in the attached memorandum from D. Soderberg to M. Hartman, June, 2000, D263890.

Three data sets are available for estimating residues on fresh apples: PDP data for analysis of individual single apples; PDP “decomposited”

apple data; and NFS "decomposited" apple data. Use of each of these three data sets for fresh apples leads to a different exposure estimate. The dietary exposure analysis has been performed using all commodities having chlorpyrifos uses and each of the apple data sets separately: PDP data for single apples; PDP "decomposited" apple data; and NFS "decomposited" apple data.

In 1999 PDP collected data on residues of chlorpyrifos on individual single apples. A total of 377 single apple samples were analyzed. Of these, 75 (20%) had measurable chlorpyrifos residues, ranging from 0.005 to 0.54 ppm. In an acute exposure analysis, results of analyses on single items of produce for a non-blended food are generally preferable to analyses of composite samples because they can be used without decompositing.

During 1994 - 1997, PDP also collected a total of 1908 composite apple samples, of which 425 samples (22%) had measurable chlorpyrifos residues, ranging from the ½ LOD for each laboratory (average 0.0026 ppm) to 0.4 ppm. Because fresh apples are considered to be a non-blended commodity, these results were decomposited using the Allender method (Allender, H. "Use of the Pesticide Data Program (PDP) in Acute Dietary Assessment", August 1998) to estimate single serving acute exposure.

DAS also submitted a market basket survey for fresh apples. All composite samples were collected from Fall 1993 - Fall 1994. There were 200 composite samples in this survey. A total of 68 samples (34%) had measurable chlorpyrifos residues, ranging from the LOD of 0.001 to 0.052 ppm.

Other programs have also analyzed fresh apples for chlorpyrifos. The FDA Surveillance Monitoring Program analyzed 1152 fresh apples (composites) between 1993 - 1998. FDA found 151 (13%) samples with measurable residues, ranging from 0.0005 ppm to 0.31 ppm.

FDA Total Diet Study (TDS) data are also available for chlorpyrifos, and in the case of apples these data also support use of the PDP data for risk assessment purposes. Measurable residues of chlorpyrifos (> 0.001 ppm) were found in apples for 14 of the 18 TDS surveys conducted from 1991 to 1997. Residues ranged from less than 0.001 ppm to 0.103 ppm, with a mean value of 0.012 ppm. Samples analyzed in the TDS are purchased at grocery stores and prepared according to standard consumer practices prior to analysis (in the case of apples this means washing). Samples are broadly composited in that composites are formed from samples purchased in three different cities from a given geographic region.

In summation, the maximum residue level found on composite apples in the NFS data is less than the maximum found in all other monitoring

programs, including the TDS, which most closely approximates NFS sampling.

NFS data on fresh tomatoes were submitted. However, only 54 samples were collected and all samples were from FL. More extensive and recent data for fresh tomatoes are available from PDP (881 samples, collected in 1996 and 1997). As was the case for apples, the highest reported detectable residue in the PDP data (0.31 ppm) was greater than that reported in the NFS data (0.0565 ppm). PDP monitoring data also reflect the use of chlorpyrifos on imported fresh tomatoes (a significant source of fresh tomatoes). Therefore the PDP fresh tomato residue data were used exclusively in all analyses. For commercially processed tomato commodities, PDP data were used but data obtained from FL grown tomatoes and fresh imported tomatoes were excluded, as these tomatoes are not used for processing. Appropriate processing residue reduction factors were incorporated for tomato juice, puree, catsup, and paste.

Exposure (consumption x residues) was compared to the acute population adjusted doses (aPAD) of 0.0005 mg/kg/day for children and females and 0.005 mg/kg/day for all other populations. The acute dietary risk analysis estimates the distribution of single day exposures for the overall U.S. population and certain subgroups. The analysis evaluates exposure to the chemical for each food commodity.

Table 3 summarizes the acute probabilistic dietary risk estimates for the U.S. Population and most highly exposed sub-populations. At the 99.9th percentile exposure, risk estimates based on the PDP single apple data, the decomposited PDP apple data, and/or the decomposited NFS apple data, were greater than 100% of the aPAD for the following population subgroups: all infants less than one-year old; children 1-6 years old; and children 7-12 years old. Children 1-6 years old were the most highly exposed population subgroup, regardless of which data set is used for fresh apples. For children 1-6 years old, risk estimates ranged from 170% to 355% of the aPAD depending on which fresh apple data set was used. Use of PDP's 1999 single apple data resulted in the highest exposure estimates. Use of the decomposited NFS fresh apple data resulted in the lowest exposure estimates.

Because the PDP single apple data are the most recent and do not require decompositing, these data are expected to provide the most reliable exposure and risk estimates. However, no matter which of the three data sets is used for fresh apples, the critical exposure commodity (CEC) analysis indicated that residues on fresh apples were the major contributor to dietary exposure estimates for children 1-6 years old at the 99.9th percentile exposure. Residues on whole tomatoes and grapes were the next major contributors to exposure.

Various risk reduction measures were examined to reduce acute dietary exposure and risk estimates. As was previously noted, fresh apples, fresh grapes and fresh tomatoes were the major contributors to acute dietary exposure for children 1-6 years old, the highest exposed subpopulation. Risk estimates could be reduced to less than 100% of the aPAD for children 1-6 years old only with mitigated exposure for all three of these commodities.

To mitigate exposure from fresh apples, the effect of deleting the late season foliar applications was examined. Currently, chlorpyrifos can be applied to apple trees when they are dormant or later in the season as a foliar treatment (up to 8 applications, with 21 days between the final two applications, and a 28 day PHI). In contrast to apples, chlorpyrifos can only be applied to pear trees as a dormant/delayed dormant application. PDP monitoring data are available for analysis of single pears. In the dietary exposure assessment, these data were translated to apples to determine the effect of deleting the apple foliar applications. Using this comparison,

residues on apples as a result of the dormant spray application are expected to be non-detectable (i.e., not expected to exceed 0.01 ppm). As part of risk mitigation, the tolerance for apples will be reassessed at 0.01 ppm, reflecting retention of only the pre-bloom application.

An examination of the PDP monitoring data for fresh grapes indicated that imported samples contained higher residues than domestic grapes. The current domestic use pattern limits application to a directed spray soil treatment to the base of dormant vines. Residues as a result of this application scenario are expected to be non-detectable (i.e., not exceed 0.01 ppm). The higher residues found on imported samples are most likely arising from later season foliar applications. As part of risk mitigation, the tolerance grapes will be reassessed at 0.01 ppm, reflecting the current domestic use pattern.

For tomatoes, PDP monitoring data again indicated that samples containing high residues were from imported fresh tomatoes. Chlorpyrifos is currently registered for use only in Florida (the state with the largest domestic production of fresh tomatoes) and Georgia. Information obtained from grower groups in FL indicates that chlorpyrifos is not used. Therefore, to mitigate dietary exposure the chlorpyrifos use on tomatoes will be deleted (i.e., tolerances revoked).

Based on these mitigation measures, risk estimates for all population subgroups are less than 100% of the aPAD as shown on Table 3. Children 1-6 years old remain the most highly exposed sub-population at 82% of the aPAD.

Table 3
Summary of Chlorpyrifos Acute Dietary Probabilistic Exposure and Risk Analysis (99.9th percentile)

Population Subgroup	PDP single apple monitoring data from 1999		"decomposited" PDP monitoring results for apples collected from 1994-1997		"decomposited" NFS monitoring results for apples collected from 1993-1994		Assuming Risk Mitigation (apples, tomatoes and grapes)	
	Exposure (mg/kg/day)	% aPAD (a)	Exposure (mg/kg/day)	% aPAD (a)	Exposure (mg/kg/day)	% aPAD (a)	Exposure (mg/kg/day)	% aPAD (a)
US Population	0.000790	16	0.000602	12	0.000453	9.1	0.000240	4.8
All Infants (< 1 year old)	0.000648	130	0.000548	110	0.000517	100	0.000258	52
Children 1-6 years old	0.001779	355	0.001247	250	0.000855	170	0.000410	82
Children 7-12 years old	0.001288	258	0.000939	190	0.000607	120	0.000319	64
Females 13-50 years old	0.000635	127	0.000484	97	0.000375	75	0.000201	40
Males 20+ years old	0.000580	12	0.000456	9.1	0.000359	7.2	0.000205	4.1

(a) The acute population adjusted dose (aPAD) is 0.0005 mg/kg/day for females and children and 0.005 mg/kg/day for all other sub-populations. Values rounded to two significant figures.

The uncertainties in the acute dietary exposure estimates are discussed below following the chronic dietary exposure assessment discussion.

4.3.2 Chronic Dietary Exposure Assessment

A refined chronic exposure analysis was performed using the DEEM ™ exposure modeling software. The input values included the PDP, FDA and DAS' NFS data, in addition to average residues from field trials and percent of the crop treated information from BEAD. All NFS data available were used except for fresh apples and tomatoes, for which PDP monitoring data were used. An additional analysis was conducted using NFS data for apples. Exposure (consumption) was compared to the chronic population adjusted dose (cPAD) of 0.00003 mg/kg/day for females and 0.0003 mg/kg/day for all other subpopulations. A summary of the residue information included in this analysis can be found in the attached memorandum from D. Soderberg to M. Hartman, June, D263889.

As shown in Table 4, for both risk estimates based on PDP or NFS data for fresh apples, the average chronic dietary residue contributions with or without the food handling establishment use are less than 100% of the cPAD and thus do not exceed HED's level of concern. Based on PDP monitoring data for fresh apples, without consideration of the food handling establishment use, the average exposure estimates comprised 3% and 61% of the cPAD for the general population and the most highly exposed subgroup, children 1-6 years old, respectively. The average exposure estimates including the food handling establishment use comprised 4% and 81% of the cPAD for the general population and for the most highly exposed subgroup, children 1-6 years old, respectively.

For the dietary exposure analysis using NFS fresh apple data, dietary risk estimates ranged from 3% to 57% for the general population and children 1-6 years of age, respectively without the food handling establishment tolerance. With food handling establishment tolerances, the dietary risk estimates ranged from 3% to 63% for the general population and children 1-6 years of age, respectively.

The effect of the risk mitigation measures discussed above, on the chronic dietary risk estimates was examined. Based on the mitigation measures (i.e., reduction of apple tolerance to 0.01 ppm based on pre-bloom application, reduction of grape tolerance to 0.01 based on domestic use pattern, and deletion of the use on tomatoes), chronic dietary risk estimates were also reduced, as shown on Table 4. Children 1-6 years old remain the most highly exposed subpopulation, with risk estimates of 51% and 36% of the cPAD, including the FHE use or using zero residues for the FHE use, respectively.

Table 4
Summary of Chlorpyrifos Chronic Dietary Exposure Analysis(a)

Population Subgroup	Estimate w/PDP Apple Data				Estimate w/NFS Apple Data				Assuming Risk Mitigation (apples, tomatoes and grapes)			
	Excludes Food Handling Establishment Use		Includes Food Handling Establishment Use		Excludes Food Handling Establishment Use		Includes Food Handling Establishment Use		Excludes Food Handling Establishment Use		Includes Food Handling Establishment Use	
	Average exposure (Fg/kg BW/day)	% cPAD	Average Exposure (Fg/kg BW/day)	% cPAD	Average exposure (Fg/kg BW/day)	% cPAD	Average Exposure (Fg/kg BW/day)	% cPAD	Average exposure (Fg/kg BW/day)	% cPAD	Average Exposure (mg/kg BW/day)	% cPAD
US Population	0.008	3	0.012	4	0.008	3	0.008	3	0.004	1.4	0.008	2.5
All infants (< 1 yr)	0.007	23	0.014	45	0.007	24	0.008	28	0.003	11	0.01	33
Children (1-6 years)	0.018	61	0.024	81	0.017	57	0.019	63	0.009	31	0.015	51
Children (7-12 years)	0.013	45	0.018	59	0.012	41	0.014	46	0.006	21	0.011	36
Females 13-50 years	0.006	21	0.009	30	0.006	20	0.006	22	0.003	11	0.006	20

(a) Values based on DEEM output, and are based on non-rounded exposure results.

Uncertainties of Dietary Exposure Estimates

The Agency believes the risk assessment presented is the most refined to date for acute and chronic dietary exposure to chlorpyrifos. However, there are some uncertainties associated with these exposure estimates as follows:

(a) Residues were detected in PDP over several years for a number of commodities that lack chlorpyrifos tolerances (i.e., chlorpyrifos is not registered for use on these commodities). These include spinach, squash, and carrots as shown below in Table 5:

Table 5
Commodities with Detected Residues in PDP and Frequently Fed to Children that Lack Established Chlorpyrifos Tolerances

Commodity	Year	# Samples with Detections	% Samples with detections	Minimum Residue Detected (ppm)	Maximum Residue Detected (ppm)
Carrots	1994	2	0.3	0.005	0.005
	1995	6	0.9	0.005	0.019
	1996	7	1.4	0.005	0.074
Spinach	1995	46	7.5	0.005	0.11
	1996	26	5.0	0.003	0.030
	1997	11	2.1	0.005	0.026
	1998 (canned)	4	0.6	0.007	0.014
Squash	1997	4	1.8	0.005	0.005
	1998	6	1.1	0.005	0.022

Residues were also detected in celery (4 samples in 1994, 0.005 - 0.045 ppm), potatoes (1 sample in 1994, 0.024 ppm), and lettuce (1 sample in 1994 at 0.01 ppm).

The FDA Total Diet Study also contains data indicating that chlorpyrifos residues in/on spinach may occur. Measurable chlorpyrifos residues have been found on cooked spinach in 10 of 18 market basket surveys (56%) conducted from 1991 to 1997.

These residue results were not included in the Agency's dietary exposure assessment as they represent misuse of chlorpyrifos. However, because these violations have occurred over the years,

excluding them might have under-represented potential dietary exposure, especially for infants and children. Therefore, an additional set of dietary exposure assessments have been performed including results for squash, spinach and carrots - three commodities frequently fed to infants and children. Celery, lettuce and potatoes were not included. These additional assessments were not significantly different from the mitigated acute or chronic dietary assessments.

(b) The consumption database used in the dietary exposure analysis (CSFII, 1989-1992) has a limited number of individuals in the age group infants less than one year old (approximately 100). The USDA is currently conducting the Supplemental Children's Survey (approximately 5000 children).

(c) The dietary exposure analyses relied primarily on monitoring data obtained either "at the farmgate" in the case of FDA or in regional distribution warehouses for PDP data. The NFS results are for samples obtained at supermarkets, but only represent one year of data. Residues potentially present on items purchased at roadside produce stands or farmer's markets are not represented in this analyses.

(d) The acute dietary analysis does not include FHE use, in accordance with current policy.

(e) Potential exposure to chlorpyrifos residues from consumption of fish was not addressed. No tolerances for fish are currently established. In 1992 the Agency's Office of Water (OW) published a report (EPA 1992) that summarized chlorpyrifos residues found in freshwater fish in lakes and rivers at that time. The primary focus of the study was monitoring for dioxin/furan in fish. However, chlorpyrifos residues were detected in 26% of the 388 sites tested, with median, mean, and maximum concentrations of non-detect, 4.09, and 344 ppb respectively. This study indicated that consumption of freshwater fish (i.e., sport fisherman and their families, or others) could contribute to dietary exposure to chlorpyrifos. FDA also has monitored farm-raised fish for chlorpyrifos. Of all fish and crustacean samples tested between 1992 to 1998, FDA found residues of chlorpyrifos in one trout (1994) and twelve catfish (four catfish in each year 1992 - 1994). FDA has found no detectable residues of chlorpyrifos in any farm-raised fish from 1995 to 1998. This is discussed in more detail below.

Chlorpyrifos Screening-Level Exposures and Risks from Freshwater Fish Consumption

In 1992, the EPA Office of Water (OW) published a report that summarized the chlorpyrifos residues in freshwater fish, and evaluated the health risks to individuals that consume freshwater fish as part of a National Screening Assessment (EPA 1992). The results of the EPA OW Assessment were not included in HED's dietary analysis because of the screening-level nature of this investigation (i.e., limited fish samples collected in areas of chlorpyrifos use, and a greater focus on bottom feeding fish such as carp and white sucker that do not contribute significantly to the diet). Nevertheless, this study indicates that consumption of freshwater fish could also contribute to the dietary exposures and risks of chlorpyrifos for sports fisherman and their families. The results of this assessment are presented below.

In the OW study, game and bottom feeding fish were collected from 388 sites, of which 314 were near point and non point sources of pollution, 39 locations were from the U.S. Geological Survey (USGS) National Stream Quality Accounting Network (NASQAN), and 35 locations represented background levels. The selection of sites was biased toward sites where dioxin/furan concentrations in fish are expected (i.e., near pulp and paper mills and industrial sources), because the original intent of study was to investigate these compounds. Consequently, few of the sites (n=15) investigated were near agricultural areas, where chlorpyrifos use is pervasive.

Chlorpyrifos was detected in fish from 26 percent of the 388 sites, with median, mean and maximum concentrations of non detect, 4.09 and 344 Fg/kg (ppb), respectively. (The second highest concentration was 64.5 Fg/kg). Over 70 percent of the fish concentrations at all sites were below detection. The highest concentrations were observed primarily in bottom feeding fish such as carp near agricultural facilities. The mean concentration from agricultural areas was 24.46 Fg/kg. In general, chlorpyrifos concentrations were detected in whole-body samples of bottom feeders and in fillet samples of game fish at roughly the same average concentration.

Health risks were calculated using fillet samples of game fish collected from 106 sites. Risk estimates were calculated using standard EPA risk assessment procedures, an average fish consumption rate of 6.5 g/day for the U.S. population, daily fish consumption over a lifetime of 70 years, and the chlorpyrifos RfD on EPA's Integrated Risk Information System (IRIS) of $3x10^{-3}$ mg/kg/day (which is an order of magnitude higher than the RfD developed by HED). The resulting hazard indices associated with ingestion of the maximum and mean chlorpyrifos fillet concentrations were

$2.4x10^{-3}$ and $6.4x10^{-5}$, respectively for the U.S. population. These risk estimates are both < 1% of the EPA RfD on IRIS, and would represent 24% and < 1% of the HED chronic PAD, respectively for chronic consumption of the maximum and mean fillet concentrations. However, it is unlikely that an individual would chronically consume the maximum detected residue of 344 Fg/kg, therefore, it may be more appropriate to compare this dose estimate to the acute PAD than the chronic PAD. In this case, consumption of fish containing 344 Fg/kg reflects only 1.4% of the aPAD.

The potential chlorpyrifos exposures could be higher for Native Americans or other subsistence populations that typically consume more freshwater fish than the general U.S. population. USEPA (1997) reports average and 95th percentile fish consumption rates of 70 g/day and 170 g/day, respectively for Native American Subsistence Populations. Consequently, potential exposures and risks could be 11 to 26 times higher than those reported for the general population of sport fisherman and their families. Risk estimates could potentially exceed HED's level of concern if chlorpyrifos fish fillet residues of 344 Fg/kg were ingested daily for 70 years at rates of 70 to 170 g/day. However, subsistence populations are not expected to have exposures or risks that exceed HED's level of concern following chronic ingestion of fish fillets with mean chlorpyrifos concentrations of 4.08 Fg/kg (up to 26% of the aPAD).

4.3.3 Drinking Water Exposure

The Environmental Fate and Effects Division (EFED) conducted a drinking water assessment for chlorpyrifos based on an analysis of existing ground and surface water monitoring data in conjunction with conservative Tier 1 and Tier 2 modeling (using GENEEC 1.2, PRZM 2.3-EXAMS, and SCI-GROW) (Attached memo from H. Nelson to D. Smegal/M. Hartman, October 6, 1999 and M. Barrett to S. Knizner, November 13, 1998). The drinking water exposure estimates are discussed in greater detail below by water source.

The available environmental fate data suggest that chlorpyrifos has a low potential to leach to groundwater from most typical agricultural uses in measurable quantities, except following termiticide use. Chlorpyrifos is persistent in concentrated applications used in termiticide treatments. The available data indicate that the primary metabolite of chlorpyrifos, 3,5,6-TCP is more mobile, and significantly more persistent in many soils, especially under anaerobic conditions.

Currently, HED uses Drinking Water Levels of Comparison (DWLOCs) as a surrogate to capture risk associated with exposure to pesticides in drinking water. A DWLOC is the concentration of a pesticide in drinking water that would be acceptable as a theoretical upper limit in light

of the total aggregate exposure to that pesticide from food, water, and residential uses. HED uses DWLOCs in the risk assessment process as a surrogate measure of potential exposure associated with pesticide exposure through drinking water. In the absence of reliable monitoring data for a pesticide, the DWLOC is used as a point of comparison against the conservative estimated environmental concentrations (EECs) provided by computer modeling (SCI-GROW, GENEEC, PRZM/EXAMS). A DWLOC may vary with drinking water consumption patterns and body weights for specific subpopulations.

HED back-calculates DWLOCs by a two-step process: exposure [food + (if applicable) residential exposure] is subtracted from the PAD to obtain the maximum exposure allowed in drinking water; DWLOCs are then calculated using that value and HED default body weight and drinking water consumption figures. In assessing human health risk, DWLOCs are compared to EECs. When EECs are **greater** than DWLOCs, HED considers the aggregate risk [from food + water + (if applicable) residential exposures] to exceed HED's level of concern.

4.3.3.1 Groundwater Exposure Levels

EFED conducted an analysis of over 3000 filtered groundwater monitoring well data available in U.S. Geological Survey's National Water Quality Assessment (NAWQA) Program databases, and in EFED's Pesticides in Ground Water Data Base (PGWDB). Chlorpyrifos was infrequently detected in groundwater (< 1% of the 3000 wells). The majority of concentrations were reported to be <0.01 Fg/L, with only occasional contamination at a maximum level of 0.026 Fg/L. Although the available monitoring data represent a large part of the U.S., it is not clear that they represent the most vulnerable groundwater where chlorpyrifos is used most intensively. The Pesticides in Ground Water Database (PGWDB) reports a maximum detected concentration of 0.65 Fg/L.

EFED also performed screening-level model estimates of chlorpyrifos concentrations in groundwater using SCI-GROW for four crops (corn, cotton, alfalfa and citrus). The estimated chlorpyrifos concentrations in groundwater using the SCI-GROW screening model range from 0.007 Fg/L (typical application to alfalfa) to 0.103 Fg/L (maximum multiple applications to sweet corn). Therefore, based on an analysis of both monitoring and modeling data, EFED concludes the large majority of the country (>99%) will not have potable groundwater that contains chlorpyrifos at levels greater than 0.1 Fg/L. EFED recommends a range of 0.007 to 0.103 Fg/L as conservative EECs to be used to evaluate both acute and chronic exposures. The

NAWQA monitoring data support that the SCI-GROW modeling estimates are conservative.

Chlorpyrifos use as a termiticide is significant, with a recent estimate of seven million pounds ai applied annually constituting about 30% of the total annual use. Chlorpyrifos groundwater exposure from termiticidal use is highly localized and usually only in wells located within 100 feet of the treatment area. For this use, the maximum detected dissolved concentration is 2090 Fg/L with unknown chronic exposure levels that are presumably significantly lower, but that can persist at detectable levels for at least 6 months. EFED recommends an upper bound range of 30 to 2090 Fg/L to evaluate acute groundwater exposures following termiticide use. The 30 Fg/L represents the concentration that DAS recommends before resuming the use of a contaminated well (i.e., current USEPA Health Advisory for a child), while the 2090 Fg/L concentration represents the maximum detected value. EFED recommends a range of 8.3 to 578 Fg/L to be used to evaluate upper bound chronic groundwater exposures for termiticide use. These values are the acute groundwater termiticide concentrations with adjustments for partial environmental degradation (abiotic hydrolysis at pH 7). DAS states that this exposure only occurs in homes where the well casing has a crack in it, and the well is near or in the foundation. HED has determined that the Label Improvement Process for Termiticides (PR notices 96-7 for termiticides) have reduced the potential for this exposure. For example, reported incidents associated with termiticide use were 28.2 per 100,000 homes in 1997 (pre PR-96-7), and were 8.3 per 100,000 homes in 1998 (post PR-96-7).

4.3.3.2 Surface Water Exposure Levels

EFED conducted an analysis of over 3000 samples from 20 NAWQA study units for flowing surface water collected from rivers and streams over the last several years. Chlorpyrifos was detected at frequencies up to 15% of 1530 agricultural streams, 26% of 604 urban stream samples in 1997 and in 65% of 57 urban stream samples from Georgia, Alabama and Florida in 1994. The maximum reported dissolved chlorpyrifos concentration in surface water was 0.4 Fg/L, with the majority of detected concentrations < 0.1 Fg/L. EFED notes that although the available monitoring data represent a large part of the U.S., the monitoring data may not represent the most vulnerable watersheds where chlorpyrifos use is pervasive. EFED notes that a limited number of watersheds in the U.S. may have chlorpyrifos concentrations higher than 0.4 Fg/L due to higher usage rates or greater pesticide runoff. In particular, acute exposure levels could be higher for streams draining watersheds with more intense chlorpyrifos use or for lakes and reservoirs for which there are little data.

EFED also performed screening-level model estimates of chlorpyrifos concentrations in surface water such as lakes and reservoirs using Tier I GENEEC or Tier II PRZM/EXAMS. Inputs to the models included high exposure agricultural scenarios for major crops (alfalfa, corn, citrus, and tobacco) at the maximum application rates. Estimated maximum 90 day average and peak concentrations of chlorpyrifos in surface water using the PRZM/EXAMS screening model were 6.7 Fg/L and 40.6 Fg/L, respectively. These estimated concentrations should be highly conservative for most surface waters and all drinking water because they are based on a pond draining an adjacent 100% treated field model (it is highly unlikely that 100% of a watershed constituting a major drinking water source would be treated with chlorpyrifos in a given year).

Based on an analysis of the NAWQA monitoring and EFED modeling data, an upper-bound EEC range of 0.026 to 0.4 Fg/L was selected to assess acute risks associated with non-termiticide uses of surface water. The 0.026 Fg/L concentration represents the 95th percentile dissolved concentration, while the 0.4 Fg/L concentration is the maximum detected dissolved chlorpyrifos concentration from streams and rivers reported in the first phase of the NAWQA study. The 95th percentile concentration of 0.026 Fg/L was used to assess chronic surface water exposures. The Agency concluded that the 0.4 Fg/L estimate (a high acute exposure level for streams) is more reasonable than the conservative PRZM/EXAMS maximum peak EEC of 40.6 Fg/L for lakes and reservoirs. This is because multi-month or annual mean concentrations in a reservoir are expected to be less than the maximum reported concentrations in the flowing water feeding the reservoir. The monitoring data also demonstrate that chronic concentrations of chlorpyrifos are unlikely to exceed 0.1 Fg/L. These estimates only apply to drinking water because residues of lipophilic pesticides, such as chlorpyrifos, bound to sediment and suspended solids could contribute to exposure following consumption of unfiltered water.

4.3.3.3 Drinking Water Exposure Concentrations

The estimated environmental concentrations (EECs) are shown on Table 6. As noted previously, the groundwater EECs are based on conservative modeling, with support from monitoring data, while the surface water EECs are based on upper-bound levels from monitoring data.

Table 6 ESTIMATED ENVIRONMENTAL CONCENTRATION (EECs)		
Drinking Water Source	**Concentration (Fg/L)**	
	Acute	**Chronic**
Groundwater, except for well contamination SCI-GROW (Fg/L) (a)	0.007 to 0.103	
Groundwater as a result of well contamination (Fg/L)	30 to 2090	8.3 to 578
Surface Water Monitoring Data (Fg/L)	0.026 to 0.4 (b)	0.026 (c)

(a) SCI-GROW (Screening Concentration in Ground Water) is an empirical model for predicting pesticide levels in ground water. The value from SCI-GROW is considered an upper bound concentration estimate.

(b) Based on the 95th percentile and maximum detected surface water concentrations.

(c) Based on the 95th percentile surface water concentration from monitoring data

In comparison, the one-day, 10-day, and longer-term USEPA health advisories for a 10-kg child are 30 Fg/L. The lifetime health advisory for a 70-kg adult has been established at 20 Fg/L; the adult longer-term health advisory is 100 Fg/L.

EFED notes that there are significant uncertainties associated with the EECs which are as follows:

(1) The estimates are intended to be as realistic as possible but apply only to the most vulnerable populations because existing monitoring data imply that the majority of the U.S. population will not be exposed at these levels (for surface water note that the 95th percentile estimate is 15 times less than the maximum detected value in monitoring data);

(2) All of these estimates are for unfinished water, and could be lower in finished drinking water that has received treatment; and

(3) The exposure estimates are highly conservative (i.e., exceed actual exposure by several-fold) for the majority of the U.S. population, based on the existing monitoring database, which covers a large part of the U.S. However, chlorpyrifos residues in surface waters could be higher in some areas where chlorpyrifos usage is more pervasive in the watershed.

4.3.3.4 DWLOCs for Acute (Drinking Water) Exposure

Acute DWLOCs were not calculated for chlorpyrifos initially because the acute dietary risks alone exceed HED's level of concern based on currently registered uses. Therefore, in effect, the DWLOCs would be zero. However, acute DWLOCs were calculated based on risk mitigation measures that reduce the acute dietary risk estimates to below 100% of the aPAD.

The acute DWLOC values are presented in Table 7. For each population subgroup listed, the acute PAD and the acute dietary (food) exposure (from Table 3) for that subgroup were used to calculate the acute DWLOC for the subgroup, using the formulas in footnotes of Table 7. The EECs are less than the DWLOCs for all populations (highest EEC of 0.4 Fg/L is less than the lowest DWLOC of 0.9 Fg/L), indicating that acute food and drinking water exposures (except possible well contamination) do not exceed HED's level of concern. It should be noted that neither the SCI-GROW model nor the monitoring data reflect concentrations after dilution (from source to treatment to tap) or drinking water treatment.

Table 7
DWLOCs for Chlorpyrifos Acute Dietary Exposure
Considering Mitigation Measures

Population Subgroup (a)	Acute PAD (Fg/kg/day)	Food Exposure 99.9th (Fg/kg/day) (b)	Max. Water Exposure (Fg/kg/day) (c)	Surface Water (Monitoring Data) (Fg/L)	Ground Water SCI-GROW, (excluding well contamination) (Fg/L)	Acute DWLOC (Fg/L) (d,e,f)
U.S. Population	5	0.24	4.76	0.026 to 0.4	0.007 to 0.103	166
All Infants (< 1 Year)	0.5	0.258	0.242			2.4
Children (1-6 years)	0.5	0.410	0.09			0.9
Females (13-50 years)	0.5	0.201	0.299			9

(a) In addition to the U.S. population (all seasons), the most highly exposed subgroup within each of the infants, children, female groups is listed.
(b) 99.9th percentile exposure. Values are from Table 3 (and rounded).
(c) Maximum Water Exposure (Fg/kg/day) = Acute PAD (Fg/kg/day) - [Acute Food Exposure (Fg/kg/day)].
(d) DWLOC (Fg/L) = Maximum water exposure (Fg/kg/day) x body wt (kg) ÷ water consumed daily (L/day)]
(e) HED default body weights are: general U.S. population, 70 kg; adult females, 60 kg; and infants/children, 10 kg.
(f) HED default daily drinking water rates are 2 L/day for adults and 1 L/day for children.

Acute exposure to chlorpyrifos in groundwater as a result of well contamination from termiticide use could potentially result in exposures of concern. However, as noted previously, the groundwater exposures from well contamination resulting from termiticide use are highly localized. The implementation of PR 96-7 for termiticides has reduced reported incidents of groundwater contamination resulting from termiticide treatments. For example, reported incidents associated with termiticide use were 28.2 per 100,000 homes in 1997 (pre PR-96-7), and were 8.3 per 100,000 homes in 1998 (post PR-96-7).

4.3.3.5 DWLOCs for Chronic Drinking Water Exposure

The chronic DWLOC is effectively zero because the long-term residential postapplication risks alone exceed HED's level of concern. However, DWLOCs were calculated based on food (including food handling establishment uses) and water exposure alone. The chronic DWLOC values are presented in Table 8. For each population subgroup listed, the chronic PAD and the chronic dietary (food) exposure (from Table 4) for that subgroup were used to calculate the chronic DWLOC for the subgroup, using the formulas in footnotes of Table 8. As shown, the EEC for surface water (which represents the 95th percentile concentration from monitoring data) is less than the DWLOCs, and therefore does not exceed HED's level of concern. It should be noted that neither the SCIGROW model nor the monitoring data reflect actual drinking water concentrations after dilution (from source to tap) or drinking water treatment.

Table 8
DWLOCs for Chlorpyrifos Chronic Dietary Exposure
Includes Mitigation

Population Subgroup (a)	Chronic PAD (Fg/kg/day)	Chronic Food Exposure with FHE (Fg/kg/day) (b)	Max. Water Exposure (Fg/kg/day) (c)	Surface Water Monitoring Data (Fg/L)	Ground Water SCI-GROW (excluding well contamination) (Fg/L)	Chronic DWLOC (Fg/L) (d,e,f)
U.S. Population	0.3	0.008	0.292	0.026	0.007 to 0.103	10
All Infants (< 1 Year)	0.03	0.01	0.02			0.2
Children (1-6 years)	0.03	0.015	0.015			0.15
Females (13-50 years)	0.03	0.006	0.024			0.72

(a) In addition to the U.S. population (all seasons), the most highly exposed subgroup within each of the infants, children, female groups is listed.
(b) Values are from Table 4 (and rounded).
(c) Maximum Water Exposure (Fg/kg/day) = Chronic PAD (Fg/kg/day) - [Chronic Food Exposure + Chronic Residential Exposure (Fg/kg/day) (if applicable)]. Chronic residential uses were not considered based on mitigation options.
(d) DWLOC (Fg/L) = Maximum water exposure (Fg/kg/day) x body wt (kg) ÷ water consumed daily(L/day)]
(e) HED default body weights are: general U.S. population, 70 kg; adult females, 60 kg; and infants/children, 10 kg.
(f) HED default daily drinking water rates are 2 L/day for adults and 1 L/day for children.

Long-term exposure to chlorpyrifos as a result of well contamination from termiticide use could potentially result in exposures of concern. However, as noted previously, the groundwater risk estimates from well contamination resulting from termiticide use are highly localized. The implementation of PR 96-7 for termiticides has reduced the reported incidents of groundwater contamination resulting from termiticide treatments.

4.4 Non-Dietary Exposure

Chlorpyrifos is an organophosphate insecticide used extensively in residential settings by both residents and PCOs, and for agricultural use (e.g., citrus, vegetable crops, tree fruits, etc.), greenhouse uses, outdoor ornamental uses, and sodfarm uses. It is one of the top five insecticides used in residential settings. There are approximately 800 registered products containing chlorpyrifos on the market (REFs 9/14/99). Registered uses include a wide variety of food, turf and ornamental plants, as well as indoor products, structural pest control, and in pet collars. It is used in residential and commercial buildings, schools, daycare

centers, hotels, restaurants, hospitals, stores, warehouses, food manufacturing plants and vehicles. In addition, it is used as an adult mosquitocide. In 1998, the DAS estimated that 70% of the urban chlorpyrifos use involved termite control. Approximately 11 million pounds a.i. are applied annually in non-agricultural settings (i.e., residences, schools, golf courses, parks).

Chlorpyrifos, is formulated as a wettable powder packaged in water soluble packets (containing 50% a.i.), emulsifiable concentrates (41.5-47%), dust (containing 0.1-7% a.i.), granular (containing 0.075%-15% a.i.), bait (containing 0.5% a.i.), flowables (containing 30% a.i.), impregnated material (containing 0.5-10% a.i.), pelleted/tableted (containing 0.5-1.0% a.i.), pressurized liquids (0.9-3.8% a.i.), microencapsulated (0.5-20% a.i.) and soluble concentrate/liquids (0.5 to 62.5% ai). Dry flowables and wettable powder in open bags are not supported by the registrant, and therefore, the assessment of these formulation types/packaging is not included in this document. According to DAS, formulations with concentrations greater than one pound a.i. per gallon (approximately 13% a.i.) are sold to licenced pest control or turf and ornamental professionals only. Lower concentrations are available to homeowners from other suppliers for over-the-counter purchase. Except aerosols, granules and dusts, all formulations for application are diluted in water to a concentration of 1 percent a.i. or less (Dow AgroSciences 1998). However, HED is aware of at least one company that sells concentrated chlorpyrifos products (i.e., >13% up to 44.8% ai) to the public on the Internet (www.ADDR.com/~pestdepo/gizhome.htm) as of March 1, 2000.

Occupational and residential exposures to chlorpyrifos can occur during handling, mixing, loading and applying activities. Occupational postapplication exposure can occur for agricultural workers during scouting, irrigation and harvesting activities. Residential postapplication exposure can occur following treatment of lawns, or residences for cockroaches, carpenter ants, termites, and other insects. In addition, there is a potential for inadvertent oral exposure to children from eating chlorpyrifos-treated turf and soil or hand to mouth activities following contact with treated surfaces or turf. Postapplication exposure to children can occur in locations other than the home, including schools, daycare centers, playgrounds, and parks. There is insufficient use information and exposure data to assess exposure resulting from use in vehicles (i.e., planes, trains, automobiles, buses, boats) and other current label uses such as treatment of indoor exposed wood surfaces, supermarkets, theaters, furniture, and draperies. However, HED has concern for these uses based on the scenarios assessed within this document, and has requested exposure data for all uses of registered products not currently assessed in this document. Although there is concern for these uses, the Agency believes that exposure from these uses will not be higher than the scenarios evaluated in this assessment.

Based on toxicological criteria and potential for exposure, HED has conducted dermal and inhalation exposure assessments for the occupational and residential handlers, occupational postapplication, in addition to residential postapplication dermal, inhalation to adults and children and inadvertent oral exposure to children.

Details of the agricultural and ornamental exposure scenarios are presented in the attached memorandum from T. Leighton to D. Smegal/M. Hartman, D263893, June 2000. Details of the occupational/residential handler assessment for residential settings and the postapplication residential risk assessment are presented in the attached memorandum from D. Smegal/T. Leighton to M. Hartman, D266562, June 2000.

4.4.1 Occupational Handler Exposure Scenarios

HED has identified 26 major exposure scenarios (resulting in 56 assessments) for which there is potential occupational handler exposure during mixing, loading, and applying products containing chlorpyrifos to agricultural crops and ornamentals (16 scenarios) and to non-agricultural use sites (10 scenarios) such as residential or recreational settings. These occupational scenarios reflect a broad range of application equipment, application methods and use sites. For agricultural uses, application techniques include tractor-drawn equipment, open and closed mixing/loading, and hand held equipment. The application rates used in the assessment are intended to reflect the upper range of rates on the labels. Maximum rates are always included in the assessment to provide a hazard evaluation for those individuals that may use the label as approved by the Agency. In some instances, the rates also include values Dow AgroSciences (DAS) specifically requested to be included as “typical” (e.g., a variety of sod farm rates, corn, citrus, greenhouse, and various nursery rates).

DAS has recently submitted a market survey (Mar-Quest) and the Agency is currently reviewing the results before including additional characterization of chlorpyrifos typical use conditions. HED also included the typical, or median use rates of 1 and 2 lb ai/acre for treatment of surface and subsurface-feeding insects on turf, respectively based on lawn care data submitted by the Registrant and TruGreen/ChemLawn (Jefferson Davis Associates, 1999, TruGreen/ChemLawn 1999). Examples of the application rates used in this assessment include, but are not limited to the following: liquid turf treatment from 1 to 4 lb ai/acre, granular turf treatment at 2 lb ai/acre, vegetable crops range from 1 to 2 lb ai/acre; maximum citrus rate is 6 lb ai/acre; the maximum rates for tree nuts and fruits is 2 lb ai/acre; outdoor ornamental rates for wettable powders are up to 4 lb ai/acre and up to 0.16 lb ai/gallon for liquid formulations; and up to 8 lb ai/acre for fire ant control in sodfarm turf just prior to harvest. The predominant maximum application

rates are defined as those rates which are most frequently cited in the labels and are also believed to be representative of the maximum allowable rates that would not underestimate exposure. Even though an attempt was made to include rates requested by DAS, some of the rates assessed do not necessarily reflect all of the typical rates used on those crops such as the tobacco rate (i.e., only maximum rate of 5 lb ai/A assessed).

The scenarios were classified as short-term (1 to 30 days), intermediate-term (1 to 6 months) and in some cases long-term (greater than 6 months) based primarily on frequency of exposure. The occupational handler scenarios for agricultural use are expected to be of a short-term duration only. It is believed that if there are any agricultural applicators applying chlorpyrifos daily for over a month, those individuals will represent a very small segment of the population. Moreover, those individuals would not be applying the amount of chemical estimated to be handled at the maximum rates in the short-term assessment. On the other hand, several of the LCO/PCO handler scenarios in residential settings (i.e., treatment of homes for insect infestations) were considered to be long-term duration. For the agricultural handlers, the estimated exposures considered personal protective equipment (PPE, which includes a double layer of clothing and gloves and/or a dust/mist respirator), and engineering controls (closed mixing/loading systems for liquids and granulars and enclosed cabs/trucks). Baseline attire (long pants, long sleeved shirt, no gloves) is not presented in this assessment to conserve resources and because of the need for additional PPE and/or engineering controls for all scenarios, and the labels currently require PPE. For LCO/PCO exposure scenarios in residential settings, in most cases only exposures associated with the label-recommended clothing were considered (i.e., scenarios with additional PPE or engineering controls could not be evaluated) based on chemical-specific studies submitted by DAS (many of which include biological monitoring).

4.4.1.1 Occupational Handler Exposure Data Sources and Assumptions

Multiple chemical-specific handler exposure studies were conducted by the registrant and submitted to the Agency. The handler data collected included biological monitoring of urinary 3,5,6-TCP, the primary metabolite of chlorpyrifos, and passive dosimetry data. These chemical-specific exposure data are used by the Agency to assess the potential handler exposures to chlorpyrifos. However, of the five agricultural monitoring studies submitted by DAS, only two of the studies measured at least 15 replicates (minimum as per the Pesticide Assessment Guideline criteria) of a specific activity (one measuring 15 replicates of both mixer/loader and airblast applicators, the other study measuring 16 replicates of a

combined mixer/loader/applicator for a granular formulation). As for the other three studies, one study measured 13 replicates of an applicator applying chlorpyrifos with various types of high pressure handwands in a greenhouse, 1 replicate of a low pressure handwand, and 2 replicates of a backpack sprayer; the second study measured 9 replicates of an open cab groundboom applicator, 6 replicates of an open mixing/loading EC formulation, and 3 replicates of an open bag WP formulation (open bag WP formulation no longer supported by DAS); and the final study measured 14 replicates of an open mixing/loading of liquids for aerial applicators. Therefore, three of the five DAS studies contain an insufficient number of replicates (as specified by Subdivision U Guidelines) to support the exposure scenarios. Moreover, the total of five agricultural studies submitted by DAS in support of the chlorpyrifos reregistration do not encompass all of the uses of the chemical on the labels nor do they all provide sufficient mitigation (e.g., PPE or engineering controls) to meet an occupational target MOE of 100.

In the absence of applicable chemical-specific data, agricultural handler and LCO/PCO potential exposures resulting from handling and applying chlorpyrifos were estimated using data from the Pesticide Handlers Exposure Database (PHED) Version 1.1 or the Draft Residential SOPs. PHED was designed by a Task Force of representatives from the U.S. EPA, Health Canada, the California Department of Pesticide Regulation, and member companies of the American Crop Protection Association. PHED is a software system consisting of two parts -- a database of measured exposure values for workers involved in the handling of pesticides under actual field conditions and a set of computer algorithms used to subset and statistically summarize the selected data. Currently, the database contains values for over 1,700 monitored individuals (i.e., replicates). HED's policy is to supplement chemical-specific data with available surrogate data in PHED to increase the sample size (U.S. EPA and HC 1995a - PHED V1.1 Evaluation Guidance). This policy is in effect because individual chemical-specific studies, even when fulfilling the Guideline minimum number of replicates, do not necessarily encompass the variety of equipment in use throughout the country and the large variability of exposures among handlers. While data from PHED provides the best available information on handler exposures, it should be noted that some aspects of the included studies (e.g., duration, acres treated, pounds of active ingredient handled) may not accurately represent labeled uses in all cases.

The PHED data used for the mixer/loader for lawn treatment, and granular bait application (hand, belly grinder and push-type spreader) scenarios in residential settings are representative of the chlorpyrifos uses as the surrogate data were monitored for the same uses.

Potential exposures and internal doses were calculated using unit exposures (i.e., normalized to amount of active ingredient handled -- mg/lb ai handled) from both passive dosimetry and biological monitoring data extrapolated to be representative of the maximum rates on the label (in some instances to typical rates). The normalized exposure data are extrapolated by multiplying by the amount of chlorpyrifos handled per day (i.e., lb ai/day). The amount of chlorpyrifos assumed handled per day was derived from the various application rates and the number of acres (or gallons of spray solution) that could be applied in a single day. Dermal and inhalation margins of exposure (MOEs) are presented separately along with a combined total MOE.

4.4.1.2 Occupational Handler Risk Characterization

A summary of the short- and intermediate-term risks estimates for PPE and engineering controls is presented in Table 9 for agricultural uses. Table 9 also provides a summary of the range of application rates assessed for chlorpyrifos. Table 10 presents a summary of the short-, intermediate, and long-term risk estimates for LCOs/PCOs at non-agricultural use sites, such as residential and recreational settings.

MOEs for occupational handlers were derived by dividing the appropriate NOAEL, shown on Table 2, by the daily dermal or inhalation exposure estimate. As noted previously, the short-term dermal NOAEL of 5 mg/kg/day is from a dermal rat study, and therefore, no dermal absorption adjustment is necessary. However, both the intermediate- and long-term dermal NOAELs of 0.03 mg/kg/day are based on the weight of evidence from 5 oral toxicity studies in dogs and rats for plasma and red blood cell cholinesterase inhibition, and consequently, dermal exposures were adjusted to absorbed dermal doses using an 3% dermal absorption factor. Inhalation exposure estimates were compared directly to the short- and intermediate-term inhalation NOAEL of 0.1 mg/kg/day, and to the long-term NOAEL of 0.03 mg/kg/day based on the weight of evidence from 5 oral studies in dogs and rats, assuming inhalation absorption is 100% of oral absorption. In evaluating biomonitoring data, which represents total chlorpyrifos exposure via dermal, inhalation and oral exposure, an adjusted absorbed dermal NOAEL of 0.15 mg/kg/day was used (i.e., 5 mg/kg/day *0.03) to estimate MOEs because most

of the total exposure is from the dermal route. Details of this assumption are presented in the HIARC report (D. Smegal April 6, 2000, HED doc no. 014088). For occupationally exposed workers, MOEs >100 (i.e., 10x for interspecies extrapolation and 10x for intraspecies variability) do not exceed HED's level of concern. MOEs below this level would represent a risk concern. A total dermal and inhalation MOE was also calculated because there is a common dermal and inhalation toxicity endpoint (i.e., cholinesterase inhibition).

Agricultural and/or Ornamental/Greenhouse Uses

The results of the short-term handler assessments as shown on Table 9 indicate that only 1 of the 16 potential exposure scenarios did not provide at least one application rate with a total MOE(s) greater than or equal to 100 at either the maximum PPE (i.e., coveralls over long pants, long sleeved shirts, and chemical resistant gloves while using open systems) or using engineering controls (i.e., closed systems). There are no data, chemical-specific or surrogate, to assess 3 of the 16 scenarios. For specific details and calculations of inhalation, dermal, and total exposures and MOEs see the attached memorandum from T. Leighton to D. Smegal/M. Hartman, D263893, June 2000. In the majority of cases, it is dermal exposure rather than the inhalation exposure driving the total MOEs.

Within the other 12 scenarios, not all of the application rates/crops have MOEs greater than or equal to 100. More specifically, the total dermal and inhalation MOEs for the 12 scenarios evaluated range from 6 to 10,000. In total, 56 iterations of potential exposures and total MOEs were calculated for the various application rates. Based on the maximum level of protection (i.e., various levels of PPE or engineering controls) 2 MOEs are estimated to be less than 10; 6 MOEs are between 10 and 50; 9 MOEs between 50 and 100 and 39 of the MOEs are greater than 100. There are insufficient information (e.g., dermal and inhalation exposure data) to assess the seed treatment uses, dip applications (e.g., preplant peach root and nursery stock), and dry bulk fertilizer applications to citrus orchard floors. These scenarios are of concern given the results from the other scenarios assessed, and HED has requested data for these uses. Fourteen of the scenarios were based on data obtained from five chemical-specific studies submitted by DAS. Of the 14 MOEs calculated using the biological monitoring results, only two reach the target MOE of 100 using PPE. The test subjects' absorbed dose levels indicate the need for additional risk mitigation measures such as closed systems for loading liquids and enclosed cabs for groundboom and airblast applicators. The results and discussion for each of the 16 exposure

scenarios are presented in greater detail in attached memorandum from T. Leighton to D. Smegal/M. Hartman, D263893, June 2000.

The agricultural handler assessments are believed to be reasonable high end representations of chlorpyrifos uses. There are, however, many uncertainties in these assessments. The uncertainties include but are not limited to the following:

- extrapolating exposure data by the amount of a.i. handled or applied; and

- not all of the exposure data are of high confidence because of the lack of replicates and/or inadequate QA/QC in the studies.

These uncertainties are inherent in most pesticide exposure assessments. The conservative nature of the assessments, however, are believed to be protective of the handlers.

Occupational/Non-Agricultural Uses (e.g., Residential/Recreational Settings)

The following scenarios (by number presented on Table 10) result in total MOEs that exceed HED's level of concern (i.e., MOE less than 100 for LCOs/PCOs):

(1) Indoor Crack and Crevice Treatment by a PCO;

(2) Broadcast Turf Treatment by a LCO (intermediate and long-term applicator/ mixer/loader);

(3) Golf Course Treatments by workers (maximum label rate of 4 lb ai/acre for: mixer/loaders of liquids, and mixer/loaders and applicators for greens and tees) and typical and maximum label rates of 1 and 4 lb ai/acre for groundboom applicators);

(5) Application of Insecticidal Dust Products by a worker;

(6) Application of Granular Formulations by a LCO by hand;

(7) Application of Granular Formulations by a LCO with a belly grinder;

(8) Application of Granular Formulations by a LCO with push-type spreader;

(9) Termiticide Treatments for Pre-Construction by a PCO;

(10) Termiticide Treatments for Post-Construction by a PCO; and

(13) Mosquitocide mixer/loader or applicator for aerial applications of more than 30 days, even with engineering controls

The following scenario results in a total MOE greater than or equal to 100 that does not exceed HED's level of concern for occupational pesticide handlers in residential settings:

(2) Mixer/loader of lawn care products wearing PPE (total MOEs 100-820);

(3) Golf Course Treatments by workers (typical label rate of 1 lb ai/acre for: mixer/loaders of liquid and wettable powders, and mixer/loaders and applicators for greens and tees; maximum label rate of 4 lb ai/acre for mixer/loaders of wettable powders) (total MOEs 100-400),

(13) Workers who mix/load or apply chlorpyrifos for aerial mosquitocide applications of less than 30 days with the use of engineering controls (closed systems)(total MOEs 160-240); and

(13) Workers who mix/load or apply chlorpyrifos for ground-based fogger mosquitocide applications up to several months with the use of PPE and/or engineering controls (total MOEs 100-560).

The results of the LCO/PCO handler assessment in residential/recreational settings for short-, intermediate and/or long-term exposure scenarios indicate that most of the MOEs are less than 100, and therefore exceed HED's level of concern. Exposure for four of the scenarios were estimated based on chemical-specific biomonitoring studies submitted by DAS (i.e., indoor crack and crevice treatment, broadcast turf application, and pre- and post-construction termiticide treatment) in which the LCOs/PCOs wore label-specified PPE, or PPE in addition to that specified on the labels. Several of these studies did not represent the maximum label application rates, or only evaluated exposures for a few hours (i.e. 1-3 hours) of the work day, and consequently could underestimate exposures and risks to LCOs/PCOs. Overall, the exposures and

risks for LCOs/PCOs based on the chemical-specific biomonitoring studies are considered to be central tendency estimates because they evaluated less than a full day's exposure at the maximum label rate or they exclude accidental exposure (e.g., exposures resulting from equipment malfunction).

All risk assessments involve the use of assumptions, judgement and available reliable data to varying degrees. Often, the available data are not the ideal data for evaluating potential exposure scenarios. This results in uncertainty in the numerical estimates of risk. Consideration of the uncertainty inherent in the risk assessment process permits better evaluation of the risk assessment and understanding of the human health impacts. Risks estimates may be overestimated or underestimated to varying degrees. Table 10 characterizes the exposure and risk estimates as low-end, central-tendency and high-end based on the assumptions used in the assessment, and identifies the most significant uncertainties.

4.4.2 Occupational Postapplication Exposure Scenarios

EPA has determined that there is potential exposure to persons entering treated sites (e.g., scouts and harvesters) after application is complete. Postapplication exposure data were required during the chlorpyrifos Data Call In (DCI) of the reregistration process, since, at that time, one or more toxicological criteria had been triggered for chlorpyrifos.

4.4.2.1 Occupational Postapplication Exposure Data and Assumptions

Multiple chemical-specific postapplication exposure studies were also conducted by the registrant and submitted to the Agency. These studies included biological monitoring and passive dosimetry data, along with dislodgeable foliar residue (DFR) data. Data were submitted by DAS for sugar beets, cotton, sweet corn, almonds, pecans, apples, citrus, cauliflower, and tomatoes. The residue decline for these crops indicate that chlorpyrifos quickly dissipates in the first few days after application and then the decline is more subtle. For instance, in most of the crops monitored, the half life of chlorpyrifos for the first part of the curve [i.e., 0 to 7 days after treatment (DAT)] is less than 1 day. However, the second part of the decline curve exhibits a half life of more than 10 days using data from sampling intervals of 7 up to 43 days after treatment (DAT). Based on the initial rapid dissipation of chlorpyrifos as shown in the DFR studies, most of the crops were analyzed using the first part of the decline curve for the short-term endpoint (i.e., up to 1 month) to

establish the restricted-entry interval (REI). The second part of the decline curve was used to assess the intermediate-term duration to assure that workers exposed in treated fields for 1 to 6 months are adequately protected. If the intermediate-term MOEs at the initially assessed short-term REI were less than 100, then the intermediate-term MOEs were used to determine the appropriate length of the REI.

Specific transfer coefficients were also monitored and submitted for citrus harvesting, citrus tree pruning, cauliflower scouting, and tomato scouting. Additional transfer coefficients for other crops/activities are currently being researched by the Agricultural Reentry Task Force (ARTF). In the mean time, HED's standard values for transfer coefficients are used to estimate potential reentry exposure because the ARTF data are not available. Once available, the ARTF data may impact the REIs for tree nuts, tree fruits, and cauliflower. In addition, chemical-specific DFR data are not available for all crops that are potentially treated with chlorpyrifos. Therefore, the assessment of postapplication exposures in this document is based on a grouping of activities associated with various representative crops. The potential for dermal contact during postapplication activities (e.g., harvesting) is assessed using a matrix of potential dermal contact rates by activity and associated crops with groupings of "low", "medium", and "high". In addition to this matrix, citrus, cauliflower, tree nuts and tree fruits are assessed separately. Table 11 summarizes the crops characterized as "low", "medium", and "high".

Maintenance workers and mowers for golf courses were also considered in this assessment and were considered to contact treated turf the day of treatment for short-term durations (i.e., less than 30 days). Although the golf course workers may be working up to 12 months a year, chlorpyrifos levels on the turf will not be available for an appreciable length of time (e.g., residues declining, irrigation, mowing of the turf).

4.4.2.2 Occupational Postapplication Risk Characterization

The results of the short- and intermediate-term postapplication assessments indicate that REIs need to be established. The REIs are presented on Tables 12 and 13. The REIs range from 24 hours for the crop grouping matrix to 10 days for harvesting cauliflower. In short, REIs are 24 hours for all crops except the following: cauliflower (10 days), all nut trees (2 days), all fruit trees (4 days) and citrus (5 days). The timing of the applications are noteworthy because most

of the applications to trees are to the bark during the dormant to early season. There is insufficient information (e.g., timing of applications -- dormant/bark versus foliar treatments) and exposure data to assess postapplication activities for ornamental and soil incorporated uses. The data needed to assess these areas include ornamental dislodgeable foliar residues in greenhouses and biological monitoring data for reentry into areas with soil directed applications. Details of this assessment are presented in memorandum from T. Leighton to D. Smegal/M. Hartman, June 2000, D263893.

Postapplication risks to golf course workers during mow/maintenance activities are presented on Table 14. The short-term MOEs are above 100 (MOE 110 to 210) and therefore, do not exceed HED's level of concern, even at the maximum label rate of 4 lb ai/acre. These risks are conservative because they assume contact with golf course turf the day of treatment.

The occupational postapplication assessments are believed to be reasonable high end representations of chlorpyrifos uses. There are, however, many uncertainties in these assessments. The uncertainties include but are not limited to the following:

- extrapolating exposure and DFR data by the amount of active ingredient handled or applied;
- not all of the exposure data are of high confidence because of the lack of replicates and/or inadequate QA/QC in the studies;
- translating crop-specific DFR data to assess other crops; and
- application timing in comparison to actual potential postapplication exposure scenarios.

These uncertainties are inherent in most pesticide exposure assessments. The conservative nature of the assessments, however, are believed to be protective of the worker.

4.4.3 Residential Handler Exposure

Potential chlorpyrifos residential handler exposures can result from treatment of turf and ornamental plants, as well as indoor use (i.e., for cockroaches, carpenter ants, etc), and structural pest control (i.e., termites). Residential handler exposures to chlorpyrifos can occur via dermal and inhalation routes during handling, mixing, loading and applying activities. All residential handler exposure durations are classified as short-term (1-30 days). As noted previously, in 1997 DAS agreed to work with EPA in

limiting household consumer use to only products packaged as ready-to-use in order to minimize exposure to concentrates that require mixing.

4.4.3.1 Residential Handler Exposure Scenarios

EPA has determined that there is potential exposure to residents during application of chlorpyrifos products. Based on residential use patterns, nine major residential/non-occupational exposure scenarios (by number presented on Table 10) were identified and evaluated for chlorpyrifos:

(1) indoor crack and crevice treatment using an aerosol can;

(2) broadcast turf mixing/loading/application using either a hose end sprayer or a low pressure hand wand;

(4) application of a 0.5% ready-to-use formulated product in a screw top bottle;

(5) application of an insecticidal dust product using a shaker can or bulbous duster;

(6) application of granular formulation by hand;

(7) application of granular formulation with a belly grinder;

(8) application of granular formulation with a push-type spreader;

(11) paintbrush application to wood for an insect infestation; and

(12) treatment of ornamentals (mixing/loading/application) using a low pressure hand wand.

4.4.3.2 Residential Handler Exposure Data Sources and Assumptions

For most cases, residential handler exposure assessments were completed by HED assuming an exposure scenario for residents wearing the following attire: short-sleeved shirt, short pants, shoes and socks, and no gloves or respirator. The only exception is the application of a ready-to-use formulated product, which was evaluated based on a chemical-specific biomonitoring study in which the volunteers wore long pants. Daily unit exposure values were obtained from the Draft Standard Operating Procedures (SOPs) for Residential Exposure Assessments (December 1997) or PHED. Eight of the nine scenarios were evaluated based on data obtained from PHED.

For broadcast turf application, the area treated per day was assumed to be 0.5 acre for hose end sprayer and 1000 ft^2 for spot treatment using a low pressure hand wand or hand application of a granular formulation. Recent lawn size survey data suggest that up to 0.5 acre lawn size represents 73% of 2300 respondents, while nearly 16% of the respondents had lawn sizes that ranged from 0.57 to 1 acre (Outdoor Residential Use and Usage Survey and National Gardening Association Survey 1999). For application of the granular formulation with a belly grinder or push-type spreader, it was assumed that an average of 0.97 lbs active ingredient was handled (i.e., 0.5 acre at 2 lb ai/acre), based on a chemical-specific study of a granular formulated product and the average of 55 replicates from the studies cited in PHED for this use pattern. For a number of scenarios, multiple evaluations were conducted using application rates less than the maximum label rate, or application using different equipment or methods (i.e., ornamental treatment via low pressure hand wand and hose-end sprayer, and granular application via hand, belly grinder and push-type spreader) to assist in risk mitigation and management decisions.

4.4.3.3 Residential Handler Risk Characterization

A summary of the short-term risk estimates, method of evaluation and risk characterization/uncertainties for residential handlers is presented on Table 10. MOEs for residential handlers were derived by dividing the appropriate short-term NOAEL, shown on Table 2, by the daily short-term dermal or inhalation exposure estimate. As noted previously, the short-term dermal NOAEL of 5 mg/kg/day is from a dermal rat study, and therefore, no dermal absorption adjustment is necessary. For inhalation, the short-term

NOAEL is 0.1 mg/kg/day based on two inhalation studies conducted in rats. Evaluation of adult biomonitoring data was conducted two ways, first the total chlorpyrifos dose was compared to an adjusted dermal NOAEL of 0.15 mg/kg/day (i.e., 5 mg/kg/day * 0.03 dermal absorption), because based on available data the majority of exposure is via the dermal route. In addition, HED segregated the total biomonitoring dose into dermal, inhalation, and oral, for comparison with the route-specific toxicity endpoints.

For residential applicators, MOEs > 1000 (i.e., 10x for interspecies extrapolation, 10x for intraspecies variability and 10x for the FQPA factor) do not exceed HED's level of concern. MOEs below this level would represent a risk concern. A total dermal and inhalation MOE was also calculated because there is a common dermal and inhalation toxicity endpoint (i.e., cholinesterase inhibition).

The results of the residential handler assessment for short-term exposure scenarios indicate that all nine scenarios evaluated have total dermal and inhalation MOEs that exceed HED's level of concern defined by a target MOE of 1000. The residential handler MOEs ranged from 3 to 900 for dermal risk, from 120 to 57,000 for inhalation risk, and from 3 to 880 for total dermal and inhalation risk for the maximum, typical and even minimum label-recommended application rates. Dermal exposure contributes most to total exposure. For a number of scenarios, multiple evaluations were conducted using application rates less than the maximum label rate, or application using different equipment or methods (i.e., ornamental treatment via low pressure hand wand and hose-end sprayer, and granular application via hand, belly grinder and push-type spreader, spot treatment for crack and crevice). These additional analyses were conducted to provide information for risk mitigation and management decisions. The following scenarios (by scenario number shown in Table 10) result in total MOEs that exceed HED's level of concern (i.e., MOE < 1000) for the typical and/or maximum application rate:

(1) indoor crack and crevice treatment using an aerosol can;

(2) broadcast turf mixing/loading and application using either a hose end sprayer or a low pressure hand wand (spot treatment);

(4) Application of a 0.5% ready to use formulated product in a screw top bottle;

(5) application of an insecticidal dust product using a shaker can

or bulbous duster;

(6) application of granular formulation by hand;

(7) application of granular formulation with a belly grinder;

(8) application of granular formulation with a push-type spreader;

(11) paintbrush application to wood for an insect infestation; and

(12) mixing/loading and treatment of ornamentals using a low pressure hand wand.

As noted previously, all risk assessments involve the use of assumptions, judgement and available reliable data to varying degrees. Often, the available data are not the ideal data for evaluating potential exposure scenarios. This results in uncertainty in the numerical estimates of risk. Consideration of the uncertainty inherent in the risk assessment process permits better evaluation of the risk assessment and understanding of the possible human health impacts. Risks estimates may be overestimated or underestimated to varying degrees. Table 10 characterizes the exposure and risk estimates as low-end, central-tendency and high-end based on the assumptions used in the assessment, and identifies the most significant uncertainties.

4.4.4 Residential/Recreational Postapplication Exposures and Risks

EPA has determined that there are potential postapplication exposures to residents/individuals entering treated areas both indoors following residential/commercial/institutional treatment (i.e., homes, schools, day care centers, etc) for cockroaches, termites or other insects and outdoors following turf treatment (i.e., homes, schools, parks, playgrounds, ball fields, etc) or mosquitocide use. In addition, there is a potential for inadvertent oral exposure to children from eating chlorpyrifos-treated soil, grass and/or granules, or placing their fingers in their mouths. For residential postapplication activities, the exposure duration is expected to be short-, intermediate- and long-term (1 days to several years) depending on the scenario. Adolescent and adult golfers were considered to contact treated turf the day of treatment for short-term durations (i.e., less than 30 days). Details of this assessment are presented in a memorandum from D. Smegal/T. Leighton to M. Hartman, June 2000, D266562.

4.4.4.1 Postapplication Exposure Scenarios

HED identified a total of eleven scenarios likely to result in postapplication exposures to residents/recreational users, and quantitatively evaluated the following ten scenarios:

(1) Indoor Crack and Crevice Treatment of kitchen and bathroom (inhalation exposure in treated room);

(2) Indoor Crack and Crevice Treatment of other rooms (dermal and oral exposure from deposition in untreated room based on registrant data);

(3) Pet Collar Products;

(4) Termiticide Treatments for Basement, Plenum, Slab and Crawlspace Construction Homes;

(6) Broadcast Lawn Treatment Using a Liquid Spray;

(7) Broadcast Lawn Treatment Using a Granular Formulation;

(8) Golf Course Exposure (adolescent and adult golfer);

(9) Aerial and ground-based fogger adult mosquitocide application;

(10) Yard and Ornamental Spray Products, and

(11) Perimeter treatment of residence.

An additional scenario, insecticidal dust product use (scenario 5) was considered, but could not be quantitatively evaluated due to an absence of chemical-specific information and residential SOPs. HED requests exposure data for this, as well as all other scenarios not evaluated.

HED is in the process of revising the Residential Exposure Assessment SOPs. This process may identify specific areas of further concern with respect to chlorpyrifos and exposure to the general population. For example, some of the secondary exposure pathways that EPA is currently examining include exposures resulting from residue tracked into homes from outdoor use, indoor dust, and spray drift. In a recent study, polycyclic aromatic hydrocarbons (PAHs) that are abundant in house dust were shown to increase the toxicity of chlorpyrifos in vitro, particularly at low levels (i.e., 2-50 FM

PAHs with 1-180 nM chlorpyrifos-oxon, a metabolite of chlorpyrifos that inhibits acetyl cholinesterase) (Jett et al. 1999). Currently, there are no SOPs available to evaluate these potential exposure pathways. These scenarios however, may be evaluated in the future pending revisions to the residential SOPs.

4.4.4.2 Data Sources and Assumptions for Postapplication Exposure Calculations

HED evaluated four of the eleven residential postapplication exposures scenarios based on chemical-specific studies submitted by DAS (i.e., crack and crevice treatment of the kitchen and bathroom (1), broadcast treatment of turf with chlorpyrifos spray (6) and granules (7), and termiticide treatment (4)). Three of these studies (crack and crevice, and two lawn studies) included biomonitoring of the urinary metabolite 3,5,6-TCP, in addition to environmental measurements to quantify chlorpyrifos exposures. In the absence of chemical-specific data, the other exposures (scenarios 2, 3, 8, 9 and 11) were evaluated using the equations and assumptions presented in the Draft SOPs for Residential Exposure Assessments guidance document or revised assumptions from the SOPs to be released in 2000 (i.e., indoor crack and crevice treatment of other rooms, mosquitocide uses, golfer exposures, pet collar uses and perimeter treatments), which are generally considered to result in high-end exposure estimates, except for the crack and crevice treatment. Scientific literature studies, the AgDrift Model and assumptions from the updated and Draft Residential SOPs were used to evaluate adult mosquitocide uses.

4.4.4.3 Residential/Recreational Postapplication Risk Characterization

A summary of the postapplication risk estimates, method of evaluation, and risk characterization/ uncertainties is presented in Table 15. MOEs for residential/recreational postapplication exposures were derived by dividing the appropriate NOAEL, shown on Table 2, by the daily dermal, inhalation or oral exposure estimate. As noted previously, biomonitoring data was evaluated two ways, first the total chlorpyrifos dose was compared to an adjusted dermal NOAEL of 0.15 mg/kg/day (i.e., 5 mg/kg/day * 0.03 dermal absorption), because the majority of exposure is via the dermal route. In addition, because there is no scientifically valid method to extrapolate from adult biomonitoring data to child exposure, HED segregated the total biomonitoring dose into dermal, inhalation, and oral exposure estimates, for comparison with the route-specific

toxicity endpoints. This extrapolation was conducted only for the post application exposures from lawn treatment. For residents, the acceptable MOE is 1000 (i.e., 10x for interspecies extrapolation, 10x for intraspecies variability and 10x for the FQPA factor). MOEs below this level would represent a risk estimate of concern for the Agency. A total dermal and inhalation MOE was also calculated because there is a common dermal and inhalation toxicity endpoint (i.e., cholinesterase inhibition). For child exposures, oral exposure also contributed to the total MOE. The following scenarios result in MOEs less than 1000, or potential exposures that exceed HED's level of concern:

(1,2) Indoor Crack and Crevice Treatment of kitchen and bathroom (inhalation exposure in treated room, dermal and oral exposure in untreated room);

(3) Pet Collar Products;

(4) Termiticide Treatments for Crawlspace, Basement, Plenum and Slab Construction Homes;

(6) Broadcast Turf Treatment Using a Liquid Spray;

(7) Broadcast Turf Treatment Using Granular Formulation;

(8) Golf Course Exposure (adolescent and adult golfer) following treatment at the maximum rate of 4 lb ai/acre, and

(11) Perimeter Treatments of Residences.

In addition, by analogy, HED evaluated yard and ornamental spray products (Scenario 10) and concluded that these products result in comparable doses and short-term MOEs with the lawn care products based on label uses and application rates. Therefore, use of many of these products is likely to result in MOEs that exceed HEDs level of concern.

The following scenarios result in MOEs greater than 1000 that do not exceed HED's level of concern for post-application residential/recreational exposures:

(8) Golf Course Use (adolescent and adult golfer) following treatment at the typical rate of 1 lb ai/acre; and

(9) Aerial and ground-based fogger adult mosquitocide application.

In conclusion, seven of the nine scenarios evaluated quantitatively have MOEs that are less than 1000, and therefore exceed HED's level of concern. In addition, for post application exposure to children following perimeter applications to homes, it was estimated that more than seven hand-to-mouth events or more than 8 minutes of play on treated turf the day of treatment could result in potential exposures that could exceed the Agency's level of concern (i.e., MOE < 1000). Total MOEs for the residential postapplication exposures that exceed HED's level of concern ranged from 6 to 980. The only postapplication scenario that resulted in a MOE consistently above 1000 was from the aerial and ground-based fogger adult mosquitocide applications (MOEs are 17,000 and 29,000 for children and adults, respectively). In addition, MOEs for adolescent and adult golfers are above 1000 following treatment of golf courses at the typical, or median rate of 1 lb ai/acre (MOEs 1500-2400). A summary of the termiticide postapplication exposure and risk estimates is presented in greater detail below.

As noted previously, all risk assessments involve the use of assumptions, judgement and available reliable data to varying degrees. Often, the available data are not the ideal data for evaluating potential exposure scenarios. This results in uncertainty in the numerical estimates of risk. Consideration of the uncertainty inherent in the risk assessment process permits better evaluation of the risk assessment and understanding of the possible human health impacts. Risks estimates may be overestimated or underestimated to varying degrees. Table 15 characterizes the exposure and risk estimates as low-end, central-tendency and high-end based on the assumptions used in the assessment, and identifies the most significant uncertainties. As noted on Table 15, the exposure and risk estimates based on the chemical-specific studies are generally considered to be reasonable central-tendency estimates (i.e., arithmetic mean, or median exposure was used to calculate risk). Because three of the chemical-specific studies were conducted in adults, conservative assumptions were used to estimate child exposures. However, because adult activity patterns differ from children, i.e., hand-to-mouth activity, some of the registrant-submitted chemical-specific studies could under-estimate a child's exposure (e.g., lawn studies are not designed to reflect any potential for incidental ingestion of residues from treated turf, soil and/or granules).

An additional scenario, postapplication exposures associated with insecticidal dust product use (scenario 5) could not be quantitatively evaluated due to an absence of chemical-specific data or recommended procedures in the Residential SOPs. Nevertheless,

HED has concerns about the use of these products based on the low MOEs calculated for residents or workers that could apply dust products. HED recommends that the registrant provide additional information on the potential post-application residential exposures associated with dust products.

HED identified a number of data gaps for assessing post application exposure, and these data gaps are discussed in Section 6.0.

HED has concerns for the potential for children's exposure in the home as a result of residential and/or agricultural uses of chlorpyrifos. Environmental concentrations of chlorpyrifos in homes may result from residential uses, spray drift, track-in, or from redistribution of residues brought home on the clothing of farm workers or pesticide applicators. Potential routes of exposure for children may include incidental ingestion and dermal contact with residues on carpets/hard surfaces, in addition to inhalation of vapor and airborne particulates. There are several literature studies that quantify the levels of chlorpyrifos in household dust, indoor and outdoor air, dermal wipe (hands) and soil samples. These residues may persist and the resulting exposures are of a potential chronic nature. Currently, there are no SOPs available to evaluate potential exposures from spray drift and track-in. The Agency is currently in the process of revising its guidance for completing these types of assessments. Modifications to this assessment shall be incorporated as updated guidance becomes available. This will include expanding the scope of the residential exposure assessments by developing guidance for characterizing exposures from other sources already not addressed such as from spray drift; residential residue track-in; and exposures to farm worker children.

Termiticide Risk Characterization and Uncertainty Analysis

Because of chlorpyrifos' extensive use as a termiticide, HED has provided a detailed summary of the risks and uncertainties associated with termiticide treatments. The Agency conducted an assessment of termiticide postapplication risks based on a chemical-specific exposure study submitted by DAS. This study collected air measurements from the basement, kitchen and bedroom of 31 homes for up to 1 year following a termiticide treatment. Four types of housing structures were evaluated: basement, plenum, slab and crawlspace. Chlorpyrifos was applied according to the label-recommended rate of approximately 1% active ingredient.

The Agency calculated incremental time-weighted average (TWA) air concentrations for the entire house, assuming an individual could be in any room. Based on this assessment, risks from inhalation exposure was the primary concern. Based on the mitigation plan, the TWA concentrations were normalized to a reduced application rate of 0.5% ai. As part of risk characterization, the Agency evaluated risks for both intermediate and chronic exposures because of uncertainties in the toxicity endpoints for both durations. Details of this analysis are presented in the Occupational/Residential Handler and Post-Application Residential/Non-Occupational Risk Assessment (memo from D. Smegal/T. Leighton, June 2000, D266562). The MOEs are presented on Table 15.

Similar to the dietary assessment, children 1-6 years of age have higher potential exposures than adults, primarily because of to a higher breathing rate per body weight, and data that indicate young children spend more time at home than adults. For children, all the 90-day median MOEs are greater than 1000 (median MOEs range from 1,900 to 3,800), and therefore do not exceed HED's level of concern. However, some of the 1-year median MOEs are below 1000, and therefore exceed HED's level of concern (median MOEs range from 530 to 1,100). As shown on Table 15, the lowest 90-day and 1-year MOEs for an individual house are 440 and 270, respectively.

The median MOEs for adults were greater than 1000 for all housing types for both the 90-day and 1-year analysis, and therefore, do not exceed the Agency's level of concern (MOEs range from 1,800 to 13,000).

There are however, a number of uncertainties in the risk assessment that arise from the following sources: choice of toxicological data used to establish the inhalation toxicity endpoint, chlorpyrifos air concentrations, and exposure assumptions. The most significant uncertainties will be discussed below.

Toxicity Endpoints: There are uncertainties associated with both the intermediate and long-term inhalation NOAELs used to calculate the MOEs. The intermediate-term NOAEL of 0.1 mg/kg/day is based on two 90-day inhalation studies, in which the rats were exposed 6 hours/day, 5 days/week (nose-only) to the highest attainable vapor concentration of chlorpyrifos (287 Fg/m^3). HED could not identify an inhalation LOAEL because no adverse effects were noted at the highest dose tested. Therefore, HED selected an oral LOAEL of 0.3 mg/kg/day to use in the dose-response

assessment. The 3 fold difference between the NOAEL and LOAEL, adds an extra buffer of safety to the intermediate-term inhalation endpoint for a total MOE of at least 3000. Although the inhalation route of exposure is ideal for this assessment, the exposure regimen does not fully mimic the potentially continuous inhalation exposure for children associated with a termiticide treatment (i.e., up to 20 hours/day).

The long-term NOAEL of 0.03 mg/kg/day is based on oral animal studies that observed cholinesterase inhibition at 0.2 to 0.3 mg/kg/day (the LOAEL). HED notes that the large difference between the NOAEL and LOAEL (i.e., factor of 6.7 to 10), adds an extra buffer of safety to the long-term inhalation endpoint. Therefore, relative to the LOAEL, the MOE is actually at least 6,000 to 10,000 for a target MOE of 1000. In addition, there are significant uncertainties associated with route-to-route extrapolation due to differences in pharmacokinetics. Following oral exposure, chlorpyrifos is absorbed in the gastrointestinal tract and is transported to the liver, where it can undergo biotransformation to a potent cholinesterase inhibitor (chlorpyrifos-oxon), and be further detoxified. However, following inhalation exposure, chlorpyrifos is absorbed directly into the systemic circulation and initially bypasses the liver. These pharmacokinetic differences may play an important role in the route-specific toxicity of chlorpyrifos. In the absence of inhalation pharmacokinetic data, it is difficult to predict whether use of an oral NOAEL would over- or under-estimate inhalation risks.

Air Concentrations: There are also a number of uncertainties associated with the chlorpyrifos air concentrations used to assess termiticide risks, which affect both the 90 day and 1 year MOEs calculations. Measured chlorpyrifos air concentrations may be overestimated because of use of other chlorpyrifos-containing products. For example, more than half (55% or 17/31) of the homes in the DAS study had detectable chlorpyrifos air concentrations prior to termiticide treatment, indicating that residents may have used other chlorpyrifos products in the home, or had a previous chlorpyrifos termiticide treatment. Several studies in the scientific literature reported chlorpyrifos air concentrations up to 8 years following termiticide treatments (Wright et al. 1988, 1994). However, these studies did not control for use of other chlorpyrifos products (i.e., lawn treatment, flea control, or other indoor uses, etc) (personal communication by D. Smegal with G. Dupree 5/17/2000), and therefore, may also overestimate potential exposures and risks.

In addition, spills inside the home can contribute to higher airborne concentrations of chlorpyrifos. In the DAS study, one of the

homes had elevated basement air concentrations because of a spill. The elevated basement measurements were excluded from the analysis (i.e., only kitchen and bedroom air data were used). This is considered reasonable because spills are likely to be an infrequent occurrence, and because pest control operators (PCOs) are trained to promptly clean spills that occur during application. However, possible applicator error, unreported, undetected or unremediated spills can contribute to air concentration measurements.

The available data suggest that temperature influences indoor chlorpyrifos concentrations resulting from termiticide treatments (i.e.,warmer temperatures are associated with higher concentrations). In the DAS study, 26 of 31 homes were from the South or warm climates. Therefore, it is possible that the air concentrations used in this assessment represent high-end estimates, that could overestimate exposures for treated houses in more temperate climates.

There are uncertainties associated with the incremental TWAs air concentration calculations. Based on the mitigation plan, HED calculated the incremental TWAs by adjusting the air measurements associated with a 0.7-1% ai product application to 0.5% assuming that there is a linear relationship between percent ai and resulting air concentrations. This assumption is considered reasonable, although it could under- or over-estimate the air concentrations associated with 0.5% a.i. product application. In addition, the 1-year incremental TWA concentration may be overestimated for two basement homes, because one year air concentration measurements were not available. HED assumed the 90 day air concentration remained constant from 90 to 365 days. This assumption only impacts two basement homes (B1 and B2), both of which had 1 year MOEs less than 1000, but 90 day MOEs greater than 1000.

Exposure Assumptions. The assumptions used to estimate exposures are based on USEPA recommended values (Exposure Factors Handbook), and are designed to be conservative for the majority of the population. These estimates could be conservative for children that do not spend their entire day at home (i.e., those that attend day-care, pre-school, and/or school). This assessment assumed that children aged 1-6 years are exposed to chlorpyrifos air concentrations in a treated home for 20 hours/day, 7 days/week, for up to 1 year.

Summary: In summary, HED believes that individuals are unlikely to experience adverse health effects from termiticide use of chlorpyrifos, even though a few of the child MOEs are below 1000.

Based on the uncertainties described above, the 90 day risk estimates may be underestimated, while the 1 year risk estimates may be overestimated. Overall, HED believes that the risk estimates are bounded by the ranges presented in Table 15. As shown on Table 15, the lowest 90-day and 1-year MOEs for an individual house are 440 and 270, respectively and the highest estimates are 13,000 and 9,500, respectively. Although some MOEs are less than 1000, there is an additional 3 to 10 fold buffer because of the difference between the NOAEL and the LOAELs. In addition, a number of conservative assumptions were incorporated into these MOEs, such as assuming that all children spend 20 hours/day, 7 days/week for up to 1 year in a treated home.

Mitigation measures will further reduce exposures and risk. For example, the removal of whole house barrier treatment addressed the exposures of most concern. It is expected that the limited spot and localized treatment, and pre-construction treatments would represent less exposure and risk. Based on the mitigation plan, and best professional and scientific judgement, HED concludes that the termiticide risk does not raise a concern and that individuals are unlikely to experience adverse health effects from termiticide treatments conducted according to the label. This conclusion is based on the conservative assumptions, the risk mitigation measures, coupled with the uncertainties of the toxicity endpoints and the air measurements.

Table 9
Exposure Variables and MOEs for Agricultural Uses
(Including Non Worker Protection Standard Ornamental Uses) of Chlorpyrifos

Exposure Scenario (Scenario#)	Are Biological Monitoring Data Available? (a)	Application Rates (lb ai/acre) (b)	Daily Acres Treated (c)	Short-Term PPE MOEs			Short-Term Eng. Control MOEs		
				Dermal	Inhalation	Total	Dermal	Inhalation	Total
Mixer/Loader Exposure									
Mixing/Loading Liquids for Aerial/Chemigation Application (1a)	Yes MRID No. 44739302	1.5 cranberries, corn	350	39	56	23	78	160	52
		3.5 citrus (d)	100	59	83	34	120	240	78
Mixing/Loading Liquids for Groundboom Application (1b)	Yes MRID No. 42974501	1.5 predominant max	80	170	240	100	Target MOE reached at PPE		
		5.0 tobacco max	80	51	73	30	100	210	69
		2 Sodfarm (includes tobacco/ potatoes)	80	130	180	75	250	530	170
		4 Sodfarm	80	64	91	38	130	260	86
		8.0 sodfarm fire ants	10	260	360	150	Target MOE reached at PPE		
Mixing/Loading Liquids for Airblast Application (1c)	Yes MRID No. 43138102	2.0 predominant max such as Fruits & Nuts	40	260	360	150	Target MOE reached at PPE		
		6.0 citrus	20	170	240	100	Target MOE reached at PPE		
Mixing WP for Aerial/Chemigation Application (2a)	No	2.0 predominant max (orchards)	350	DAS is not supporting the open bag formulation for the WP			51	42	23
		3.5 citrus (d)	100				100	83	46
Mixing WP for Groundboom Application (2b)	Yes MRID No. 42974501	1.0 predominant max (brassica)	80				450	360	200
		4.0 soil treatment ornamentals outdoors	10				890	730	400
		1.3 & 3.0 Sodfarm	80				340 / 150	280 / 120	150 / 67

Table 9
Exposure Variables and MOEs for Agricultural Uses
(Including Non Worker Protection Standard Ornamental Uses) of Chlorpyrifos

Exposure Scenario (Scenario#)	Are Biological Monitoring Data Available? (a)	Application Rates (lb ai/acre) (b)	Daily Acres Treated (c)	Short-Term PPE MOEs			Short-Term Eng. Control MOEs		
				Dermal	Inhalation	Total	Dermal	Inhalation	Total
		8.0 sodfarm fire ants (harvest only)	10				4500	3600	200
Mixing WP for Airblast Application (2c)	No	2.0 predominant max	40				450	360	200
		6.0 citrus	20				300	240	130
Loading Granulars for Aerial Application (3a)	No	1.95 maximum aerial rate	350	150	30	25	3000	300	270
Loading Granulars for Ground Application (3b)	Yes MRID No. 44483501 (3b and 8)	1.0 typical corn	80	1300	260	210	Target MOE reached at PPE		
		2.0 max corn	80	640	130	110	Target MOE reached at PPE		
		3.0 maximum ground rate (tobacco)	80	430	86	71	8600	860	780
Applicator Exposure									
Aerial (Spray) -- Enclosed Cockpit (4a)	No	2.0 orchards	350	No Open cockpit data available			100	150	60
		3.5 citrus (d)	100				200	290	120
Aerial (Granulars) -- Enclosed Cockpit (4b)	No	1.95	350	No Open cockpit data available			320	8	8
Groundboom Tractor (5)	Yes MRID No. 42974501	1.5 predominant max	80	The biological monitoring results (Table A4) indicate that open cabs provide insufficient protection . Therefore, only the enclosed cab MOEs are presented.			580	1400	410
		5.0 tobacco max	80				180	410	120
		4 Sodfarms	80				220	510	150
		8.0 sodfarm fire ants	10				880	2000	610

Table 9
Exposure Variables and MOEs for Agricultural Uses
(Including Non Worker Protection Standard Ornamental Uses) of Chlorpyrifos

Exposure Scenario (Scenario#)	Are Biological Monitoring Data Available? (a)	Application Rates (lb ai/acre) (b)	Daily Acres Treated (c)	Short-Term PPE MOEs			Short-Term Eng. Control MOEs		
				Dermal	Inhalation	Total	Dermal	Inhalation	Total
Airblast Applicator (6)	Yes MRID No. 43138102	2.0 predominant max	40	The biological monitoring results indicate that open cabs are insufficient.			230	190	110
		6.0 citrus	20				150	130	70
Tractor-Drawn Granular Spreader (7)	Yes MRID No. 44483501 (3b and 8)	1.0 typical corn	80	1000	360	270	Target MOE reached at PPE		
		2.0 max corn	80	520	180	140	Target MOE reached at PPE		
		3.0 maximum ground rate (tobacco)	80	350	120	90	690	130	110
Seed Treatment (8)	No	No Data	No Data	No Data			No Data		
Dip Application (Preplant Peaches) (9)	No	No Data	No Data	No Data			No Data		
Flagger Exposure									
Spray Applications (10)	No	2.0 predominant max	350	50	140	37	2300	1400	880
		3.5 citrus (d)	100	100	290	74	4500	2900	1800
Granular Applications (11)	No	1.95	350	320	340	170	Target MOE reached at PPE		
Mixer/Loader/Applicator Exposure									
Backpack Sprayer (12)	Yes MRID No. 43027901	0.0417 lb ai/gal predominant max / 0.08 lb ai/gal bark beetle treatment / 0.03 lb ai/gal stump treatment	40 gal/day	130 / 68 / 180	700 / 360 / 970	110 / 58 / 150	Target MOE reached at PPE, except for the higher concentration for the beetle bark treatment		
		3.5 citrus bark	1 A/day	63	330	53	Not feasible		
		0.039 lb ai/gal /750 ft2	1000 ft2	4200	22000	3500	Target MOE reached at PPE		

Table 9
Exposure Variables and MOEs for Agricultural Uses
(Including Non Worker Protection Standard Ornamental Uses) of Chlorpyrifos

Exposure Scenario (Scenario#)	Are Biological Monitoring Data Available? (a)	Application Rates (lb ai/acre) (b)	Daily Acres Treated (c)	Short-Term PPE MOEs			Short-Term Eng. Control MOEs		
				Dermal	Inhalation	Total	Dermal	Inhalation	Total
Low Pressure Handwand (13)	Yes MRID No. 43027901	0.0417 lb ai/gal predominant max / 0.08 lb ai/gal bark beetle treatment / 0.03 lb ai/gal stump treatment	40 gal/day	570 / 300 / 790	700 / 360 / 970	310 / 160 / 440	Target MOE reached at PPE		
		3.5 citrus bark	1 A/day	270	330	150	Target MOE reached at PPE		
		0.039 lb ai/gal/ 750 ft2 animal prem.	1000 ft2	18000	22000	10,000	Target MOE reached at PPE		
High Pressure Handwand (greenhouse uses) (14)	Yes MRID No. 43027901	Min. 0.0033 lb ai/gal	1000 gal/day	66	88	38	Not feasible		
		Max. 0.0066 lb ai/gal		33	44	19	Not feasible		
Hydraulic Hand-held Sprayer for Bark / Pine Seedling Treatment (15)	No	3.5 citrus bark	10	16	100	14	Not feasible		
		0.08 lb ai/gal bark beetle treatment / 0.16 lb ai/ gal pine seedling treatment /	1,000	14 / 7	88 / 44	12 / 6	Not Feasible		
		0.039 lb ai/gal /750 ft2 animal prem	10000 ft2	2,200	13,000	1,900	Target MOE reached at PPE		
Dry Bulk Fertilizer Impregnation	No	1.0 lb ai / 200 lb fertilizer / acre	No Data	No Data			No Data		

(a) Biological monitoring data are available from several chemical-specific studies. Although biological monitoring scenarios are available for some of the scenarios as indicated in this table, passive dosimetry data are presented for comparison because insufficient replicates and/or additional risk mitigation measures were necessary.

(b) Application rates are the maximum labeled rates found on EPA Reg. Nos. 62719-38, -221, -245, -34; -79, -72, -166, -220, 34704-66 (Clean Crop Chlorpyrifos 4E -- sodfarm fire ant rate), 499-367 (499-367 is the only greenhouse label identified), and 10350-22 for animal premise treatments. **"Predominant max"** in this table refers to the most **frequently identified maximum** application rate found on the labels for the specific formulation and equipment type. Typical rates are also included to characterize the chlorpyrifos uses. Not all application rates are included for all crops, instead, a cross-section of rates are used to represent the uses of chlorpyrifos.

(c) Daily acres treated are based on HED's estimates of acreage (or gallonage) that would be reasonably expected to be treated in a single day for each

exposure scenario of concern. The sodfarm fire ant rate is restricted on the label for harvest only, therefore, this rate is limited to the amount of sod that may be harvested in a reasonable time frame. Therefore, using the limited data available, approximately 10 acres treated per day are assumed to be the upper range.

(d) The application rates on the Lorsban 4E (EPA Reg. No. 62719-220) and 50W (EPA Reg. No. 62719-39 discontinued as of 1995 and sold as -221) labels indicate that for citrus at the 6.0 lb ai/A rate it is necessary to use 100 to 2,400 gallons per acre dilute spray. Therefore, this rate is not expected to be feasible for an aerial applicator. The label language should be clarified so that the 6.0 lb ai/A rate is for ground only. Additionally, citrus orchards are believed to be relatively small plots and 100 acres per day is assumed in the assessment for aerial applications.

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
(1) Indoor Crack & Crevice Treatment						
Long term PCO Applicator (0.29% Dursban Pro; EPA Reg. 62719-166)	double layer clothes, chemically-resistant boots and gloves, eye protection	Biomonitoring study MRID No. 44444801 (minimum, mean and maximum amount handled)	17 (max) 59 (mean) 5900 (min)	58 (max) 200 (mean) 20,000 (min)	13 (max) 45 (mean) 4500 (min)	Central-tendency risk estimates for applicators; MOEs less than 100 for workers that could handle $0.02 lb ai/day (the mean amount handled in the study). Only two of 15 replicates reflect the maximum label concentration of 0.5% ai. (avg of 0.29% ai was handled in study). Underestimates exposure to workers that mix/load and apply chlorpyrifos because study only evaluated applicators.
Short-term Residential Applicator (EPA Reg 026693-00003 for 1% ai; 239-2619 for 0.5% ai)	SS, SP, no gloves	Residential SOPs (PHED V1.1)	159 (1%) 318 (0.5%) 2540 (spot treatment)	292 (1%) 584 (0.5%) 4700 (spot treatment)	100 (1%) 200 (0.5%) 1600 (spot treatment)	High-end risk estimates for 1% ai; central tendency for 0.5% ai; assumes application of one 16 oz. aerosol can for both; low-end to central tendency risk for spot treatment which assumes 2 oz application of 0.5% ai. product
(2) Broadcast Turf Application (Intermediate and Long-Term for PCOs; Short-Term for Residential Applicators)						
Applicator (1 or 4 lb ai/Acre of Dursban Pro, EPA Reg. 62719-166)	single layer clothes, chemically-resistant knee high boots and gloves, hat (knee high boots not required by label)	Biomonitoring Study MRID No. 44729401 (25% of label maximum rate or adjustment for label-recommended max application rate)	Biomonitoring: 75 (IT&LT) (1 lb ai/acre) Label Max: 20 (IT&LT) (4 lb ai/acre)			Central-tendency risk estimates for 1 lb ai/acre; product applied at 25% of label maximum. High-end risk estimates for 4 lb ai/acre (label maximum for subsurface soil treatment). Study evaluated an average 1.5 hour spray time over a 6 hour work day which may underestimate worker exposure based on TruGreen/ChemLawn data for 193 workers that show an average spray time of 2.75 hours over a 8.75 hour work day.
Mixer/Loader (liquid) (Dursban Pro, EPA Reg. 62719-166)	single layer clothes, gloves	PHED V1.1 (biomonitoring study rate and 25% of maximum label rate)	260-1032	500-1980 (IT) 150 -600 (LT)	170-680 (IT) 100-380 (LT)	Central-tendency to High-end risk estimates; maximum ai handled in study with maximum (4 lb ai/acre) and 25% of maximum label rate (1 lb ai/acre), respectively
	double layer clothes, gloves		350 -1400		200-820 (IT) 100 -420 (LT)	

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment						
Application Scenario	**Clothing**	**Method of Evaluation**	**MOE**			**Risk Characterization/ Uncertainties**
			Dermal	**Inhalation**	**Total**	
Residential Mixer/Loader/ Applicator Broadcast with Hose End Sprayer (Dursban 1-12 Insecticide EPA Reg 62719-56)	SS, SP, no gloves	Residential SOPs (PHED V1.1) (min and max dilution rates)	6-23	368-1470	6-23	Central-tendency to High-end risk estimates; Low confidence in exposure estimates from PHED V1.1; assumes resident handles 22 gallons of minimally and maximally diluted product
Residential Mixer/Loader/ Applicator Spot treatment with Low Pressure Handwand (Dursban 1-12 Insecticide EPA Reg 62719-56)	SS, SP, no gloves	Residential SOPs	37-150	2490-9960	37-150	Central-tendency to High-end risk estimates; Low confidence in dermal exposure estimates, and medium confidence in inhalation exposure estimates; assumes resident handles 1 gallon of minimally and maximally diluted product to treat 1000 $ft^{2.}$
(3) Golf Course Use (Dursban Turf Insecticide; EPA Reg. 62719-35) (Short-term)						
Mixer/Loader (Liquid)	LS, LP, gloves	PHED V1.1	95-380	36-150	26-100	High-end for 4 lb ai/acre and central tendency for 1 lb ai/acre; assumes handling product to treat 40 acres at 1-4 lb ai/acre. Using PHED only 4 lb ai/acre results in MOEs < 100 for liquid mixer/loader (MOE=26). For groundboom applicator, MOE < 100 based on biomonitoring at both 1 and 4 lb ai/acre. HED has more confidence in the biomonitoring results than PHED.
Mixer/Loader (Wettable Powder in water soluble bags)	LS, LP, gloves	PHED V1.1	220-820	180-730	100-400	
Groundboom Applicator	LS, LP, no gloves	PHED V1.1	160-630	59-240	43-170	
		Biomonitoring (MRID 42974501)	15-63		15-63	
Mix/Load/Apply via Handgun (greens/tees) (Liquid)	LS, LP, gloves	PHED V1.1	49-190	130-540	36-140	High-end for 4 lb ai/acre and central tendency for 1 lb ai/acre; assumes handling product to treat 5 acres at 1-4 lb ai/acre. Only 4 lb ai/acre results in MOEs < 100

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
(4) Ready-to-Use 0.5% a.i. Formulated Product (Ortho Ant Stop)						
Short-term Residential Applicator	SS, LP, no gloves	Outdoor Biomonitoring Study MRID No. 44739301	625 (biomonitoring)		625	Central-tendency to high-end risk estimate; assumes resident applies five 24 oz bottles of product/day, however, resident wore long pants and current HED policy is to evaluate exposures for short pants. Risks calculated two ways, one using total exposure based on biomonitoring, and second by comparing estimated route-specific exposure to appropriate toxicity endpoints.
			714	3,400	590	
(5) Insecticidal Dust Product (Shaker Can or Bulbous Duster)						
Residential Applicator (1% ai chlorpyrifos; 2.83 g ai) (EPA Reg. 62719-66, 62719-54, and 192-171)						
Short- term	SS, LP, no gloves	Scientific Literature Study	250	NE	250	Central-tendency to High-end risk estimates; assumes an individual applies a 10 oz can of 1% ai chlorpyrifos dust; neglects inhalation exposure due to an absence of data.
Worker (7% ai chlorpyrifos; 7.91 or 198 g ai) (EPA Reg. 13283-17, Rainbow Kofire Ant Killer)						
Short- term	LS, LP, gloves	Scientific Literature Study	98 (7.9 g) 3.9 (198 g)	NE	98 (7.9 g) 3.9 (198 g)	Central-tendency short term risk assessments for 7.9 and 198 g ai; High-end intermediate-term risk estimates for 7.9 and 198 g ai (based on size of dust container); Neglects inhalation exposure due to an absence of data.
Intermediate term			20 (7.9 g) 0.8 (198 g)	NE	20 (7.9 g) 0.8 (198 g)	

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
(6) Granular Formulation (Hand Application) (EPA Reg. 672719-14, 62719-210) (2 lb ai/acre)						
LCO (intermediate-term)	LS, LP, gloves	PHED V1.1	21	324	20	High-end risk estimates; medium confidence in PHED unit exposure estimates which are based on a single study in which a test subject wearing chemical-resistant gloves spread the granular formulation around the outside of the residence and over 90 percent of the samples contained no detectable material. Therefore, residents also evaluated wearing long pants, long sleeved shirt and gloves. Assumes treatment of 1000 ft^2. Could underestimate exposure because PHED data excludes head and neck area.
	Double layer clothing, gloves		38	324	34	
Residential Applicator (short-term)	SS, SP, no gloves	Residential SOPs	18	327	17	
	LS, LP, gloves		106	330	80	
(7) Granular Formulation (Belly Grinder) (EPA Reg. 672719-14, 62719-210) (2 lb ai/acre)						
LCO (intermediate-term)	LS, LP, gloves	PHED V1.1	8	120	7	Central-tendency risk estimates for worker; High-end risk estimates for residents, except for spot treatment. Low and high confidence in the dermal and inhalation exposure estimates, respectively. Assumes treatment of 0.5 acre at typical rate of 2 lb ai/acre for subsurface feeding insects. Could underestimate exposure because PHED data excludes head and neck area. Workers could treat more than 0.5 acre/day.
	Double layer clothing, gloves		12.5	120	11	
Residential Applicator (short-term)	SS, SP, no gloves	Residential SOPs	3	120	3	
			69 (spot)	36 (spot)	24 (spot)	
(8) Granular Formulation (Push-type Spreader) (EPA Reg. 672719-14, 62719-210)(2 lb ai/acre)						
LCO (intermediate-term)	LS, LP, gloves	PHED V1.1	57	1150	54	Central-tendency risk estimates for worker; High-end risk estimates for residents. Low and high confidence in the dermal and inhalation exposure estimates, respectively. Assumes treatment of 0.5 acre at typical rate 2 lb ai/acre for subsurface feeding insects. Could underestimate exposure because PHED data excludes head and neck area. Workers could treat more than 0.5 acre/day.
	Double layer clothing		100	1150	92	

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
Residential Applicator (short-term)	SS, SP, no gloves	Residential SOPs	120	1150	110	

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
Termiticide Treatments						
(9) Pre-Construction (1.44% chlorpyrifos as Dursban TC) (EPA Reg. 62719-47) (long-term)						
Mixer/Loader/ Applicator (3 hour average exposure)	label-specified PPE: single layer clothes and forearm-length chemically-resistant gloves (forearm length gloves not required by label)	Dosimetry and air monitoring from Registrant Study MRID No. 44589001	19	67	15	Low-end risk estimates for workers that wore double layer of clothing and forearm length gloves not required by the label; Central-tendency risk estimates for workers that wore a single layer of clothing and forearm length gloves; assumes 3 hour exposure, which could underestimate risks to workers exposed > 3 hrs/day, or that use 2% ai to treat utility poles or fences
	double layer clothes (LS,LP, coveralls, rubber boots, and forearm-length gloves) (forearm-length gloves not required by label)		63	67	33	
Tarp puller	with forearm-length gloves (LS,LP, leather and/or rubber boots and hat)	Dosimetry and air monitoring from Registrant Study (1-8 tarps) MRID No. 44589001	170-1300	180-1400	87 (8 tarps) 690 (1 tarp)	Central-tendency risk estimates; assumes workers pull 1-8 tarps/day (7 min/tarp), could underestimate risks to workers who pull > 8 tarps/day (i.e., >1 hr exposure/day). All total MOEs < 100 for 8 tarp/day. Also, workers wore forearm length gloves not required by the label which reduce estimated exposure.
	without gloves (LS,LP, leather and/or rubber boots and hat)		47-370	240-2000	39 (8 tarps) 310 (1 tarp)	
(10) Post-Construction (1% chlorpyrifos as Dursban TC) (EPA Reg. 62719-47) (long-term)						
Mixer/Loader/ Applicator	Label-specified PPE: LS, LP, chemically resistant gloves, hat, eye protection and half face piece respirator in confined spaces; During M/L: 2 layers clothes and chemically-resistant shoes	Biomonitoring: 4.3 MRID No. 44729402 (n=5)	7		7	Central-tendency risk estimate, could underestimate risks for workers that apply 2% ai to treat utility poles or fences
		Dosimetry and air monitoring MRID No. 44729402 (n=14)	12	33	9	Central-tendency risk estimate; excludes worker with higher exposure (10X greater than mean) due to a broken hose

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
(11) Paint Brush (Short-term) (Dursban 1-12 Insecticide, EPA Reg. 62719-56)						
Residential Applicator	SS, SP, no gloves	Residential SOPs; 1 gallon for worst case and 1 quart for typical case	37 (1 gal) 148 (1 qt)	590 (1 gal) 2300 (1 qt)	35 (1 gal) 140 (1 qt)	Central-tendency risk estimates for typical case and high end risk estimates for worst case; low to medium confidence in dermal exposure estimates and medium confidence in inhalation exposure estimates; Assumes resident applies 1 gallon or 1 quart of diluted product in a day
(12) Ornamental Application (Short-term) (Dursban 1-12 Insecticide, EPA Reg. 62719-56)						
Residential Mixer/Loader/ Applicator Low pressure Handwand	SS, SP, no gloves	Residential SOPs (minimum : 1 oz/3gal H20)	270	18,000	270	Central-tendency to high-end risk estimates; low and medium confidence in the dermal and inhalation exposure estimates, respectively. Assumes resident applies 5 gallons of diluted product/day.
		Residential SOPs (typical 4 oz/3 gal H20)	70	4,700	69	
		Residential SOPs (max. 1 qt/3 gal H2O)	8	560	8	
Residential Mixer/Loader/ Applicator Hose End Sprayer	SS, SP, no gloves	Residential SOPs (minimum : 1 oz/3gal H20)	900	57,000	880	Central-tendency to high-end risk estimates; low confidence in the dermal and inhalation exposure estimates. Assumes resident applies 5 gallons of diluted product/day.
		Residential SOPs (typical 4 oz/3 gal H20)	230	15,000	230	
		Residential SOPs (max. 1 qt/3 gal H2O)	28	1,800	28	
(13) Mosquitocide Mixer/Loader/Applicator (PHED V1.1) (Short- and intermediate-term) (Mosquitomist One EPA Reg. 8329-24)						
Mixer/Loader--Aerial	PPE double layer clothes and gloves	PHED V1.1	120 (ST) 24 (IT)	34 (ST&IT)	26 (ST) 14 (IT)	High end risk estimates. Application rate of 0.023 lb ai/acre for 7500 acres
	Engineering Controls (enclosed cockpit) single layer clothes and gloves		236 (ST) 47 (IT)	490 (ST&IT)	160 (ST) 43 (IT)	

Table 10. Estimates of Risks to Commercial Applicators and Residents Applying Chlorpyrifos in the Residential/Recreational Environment

Application Scenario	Clothing	Method of Evaluation	MOE			Risk Characterization/ Uncertainties
			Dermal	Inhalation	Total	
Mixer/Loader--Ground-based fogger	PPE, single layer clothes and gloves		1010 (ST) 200 (IT)	390 (ST&IT)	280 (ST) 133 (IT)	High end risk estimates. Application rates of 0.005 and 0.01 lb ai//acre for 3000 acres. Surrogate ground-based fogger exposure data are not available, and therefore, it was necessary to extrapolate from airblast exposure data
	engineering controls (enclosed cab) and single layer clothes and gloves		270 (IT)	2800 (IT)	250 (IT)	
Aerial Applicator	engineering controls (enclosed cockpit) and single layer clothes and no gloves		400 (ST) 81 (IT)	600 (ST&IT)	240 (ST) 71 (IT)	High end risk estimates. Application rate of 0.023/acre for 7500 acres
Ground-based fogger Applicator	engineering controls (enclosed cab) and single layer clothes and no gloves		610-1230 (ST)	520-1040 (ST)	280-560 (ST)	High end risk estimates. Application rates of 0.005 and 0.01 lb ai/acre for 3000 acres. Surrogate ground-based fogger exposure data are not available, and therefore, it was necessary to extrapolate from airblast exposure data
			120-250 (IT)	520-1040 (IT)	100-200 (IT)	

LS=Long sleeves; LP = Long pants; SS = short sleeves; SP = short pants
H20 = water; ST = short-term (1- 30 days); IT = intermediate term (30 days to 6 months) LT = long term (> 6 months)
NE = Not evaluated

TABLE 11 Crop Grouping Matrix by Potential for Dermal Contact			
Potential for Dermal Contact	**Transfer Coefficient (cm^2/hr)**	**Activities**	**Crops**
Low	2,500	Harvest	Alfalfa, asparagus, small grains (wheat, sorghum, milo), soybeans, cole crops, mint
		Sort/Pack	Sugar beets, radishes, rutabagas
Medium	4,000	Harvest, stake/tie, scout, irrigate	Cranberries, strawberries
		Irrigate	Christmas trees
		Late season scouting	Cotton
High	10,000	Harvest	Sunflowers, sugar beets, corn (up to 1.5 lb ai/A as a foliar treatment), sweet potatoes, radishes, rutabagas, turfgrass (sodfarm) for fire ants, almond harvesting
		Cut/harvest, prune, transplant, ball/burlap	Christmas trees

TABLE 12 Restricted Entry Intervals (REIs) for Chlorpyrifos: General				
Potential for Dermal Contact	**Short-Term REIs (days)**		**Intermediate-Term REIs (days)**	
	1 lb ai/A	**2 lb ai/A**	**1 lb ai/A**	**2 lb ai/A**
LOW	1	1	1	1
MEDIUM	1	No Crops	1	No Crops
HIGH	1	1	1	2
Scouting (Various Crops)	0	1	1	1

TABLE 13
Restricted Entry Intervals (REIs) for Chlorpyrifos:
Cauliflower, Citrus and Tree Nuts & Fruit

Activity	Short-Term REIs (days)					Intermediate-Term REIs (days)				
	Almonds	Apples	Pecans	Cauli-flower	Citrus	Almonds	Apples	Pecans	Cauli-flower	Citrus
Scouts	2	1	0	1 to 3	2	2	1	0	1 to 3	2
Harvesting	5	3	1	5 to 8	5	7	4	2	7 to 10	5
Pruning (wet cond.)	NE	NE	NE	NA	4	NE	NE	NE	NA	5
Pruning (dry cond.)	NE	NE	NE	NA	2	NE	NE	NE	NA	2

NE = Not Evaluated

Table 14
Chlorpyrifos Surrogate Occupational Postapplication Assessment for Golf Course Turf Treatment

Crop	Application Rate	DAT (a)	TTR from WP (Fg/cm^2) (b)	Mow/Maintain Transfer coefficient =500 cm^2/hr		Mow/Maintain Transfer coefficient =1,000 cm^2/hr	
				Potential Dermal Dose (mg/kg/day) (c)	Short-term MOE (d)	Potential Dermal Dose (mg/kg/day) (c)	Short-term MOE (d)
Golf Course Turf	4.0	0	0.414	0.024	210	0.047	110

(a) DAT is "days after treatment."

(b) Turf Transferable residues (TTR) from MRID 448296-01 based on average of CA, IN and MS sites following application of 4 lb ai/ Acre of Dursban 50W.

(g) Dermal Dose = TTR (Fg/cm^2) x Transfer coefficient (cm^2/hr) x conversion factor (1 mg/1,000) x 8 hr/day duration x dermal absorption x 1/70 kg body weight. The target MOE of 100 is based on 10x interspecies and 10x intraspecies.

(d) Short-term MOE = NOAEL of 5 mg/kg/day / Potential dermal dose (mg/kg/day).

Table 15. Estimates of Post-Application Risks to Residents/Recreational Users				
Reentry Scenario	**Method of Evaluation**	**Central-tendency MOE**		**Risk Characterization/ Uncertainties**
		Adult	**Child**	
(1) Crack & Crevice Treatment of Kitchen and Bathroom (0.5% Dursban Pro diluted spray, EPA Reg. 62719-166) (Short and Intermediate Term)				
Maximum 1-Day Inhalation Exposure:	Biomonitoring Study, with environmental measurements	560	130	Central-tendency to High-end risk estimates; assumes exposure exclusively through inhalation and that children spend 21 hours/day (50th percentile for 1-4 yr old at home) in a treated room (i.e., home, schools, day care centers, etc). This could over-or under-estimate risk because it is compared to a 90 day inhalation NOAEL for rats exposed 6 hours/day.
10-Day TWA Inhalation Exposure		670	360	
(2) Crack & Crevice Treatment Using Residential SOPs (0.5% Dursban Pro diluted spray, EPA Reg. 62719-166) (Short-term)				
Dermal Exposure From Carpets	Highest deposition from untreated family room in biomonitoring study (room adjacent to treatment) and Residential SOPs	1950	1360	Low-end risk estimates; highest deposition from untreated room used in conjunction with updated SOP assumptions (i.e., 5% of residues are dislodgeable, 50% extracted in saliva, transfer coefficients of 6,000 and 16,700 cm^2 for children and adults, respectively). Inadequate deposition data collected in treated rooms in registrant study.
Dermal Exposure From Surfaces		3900	2700	
Oral Exposure		NE	4100	
Total Crack &Crevice (Sum of 1 and 2) Inhalation, Dermal and Oral		390 (1 day) 440 (10day)	110 (1 day) 240 (10day)	Central-tendency risk estimates. Inhalation estimates are central-tendency to high end, but dermal and oral exposure estimates are low end.
(3) Pet Collar Uses (11 month efficiency) (Long-term)				
Dog Collar (EPA No. 45087-49; 3.44 g ai); Cat Collar (EPA No. 4306-16; 0.93 g chlorpyrifos)				
Total Exposure	Residential SOPs	670 (dog) 2500 (cat)	140 (dog) 530 (cat)	Central-tendency to high-end risk estimates; assume that a total of 1% ai is available from collar over 11 months only from dermal exposure. Assumes incidental ingestion and inhalation are negligible. Based on preliminary data, equivalent to approximately 2 , 3 or 105 min per day of vigorous dermal contact with collar, neck fur or back fur over 11 months.

Table 15. Estimates of Post-Application Risks to Residents/Recreational Users				
Reentry Scenario	**Method of Evaluation**	**Central-tendency MOE**		**Risk Characterization/ Uncertainties**
		Adult	**Child**	
(4) Termiticide Treatment Includes Risk Mitigation (adjustment to 0.5% ai as Dursban TC) (Intermediate and Long-term) (See Table A-1, Appendix A)				
Basement Construction				
90-Day Incremental Time-weighted- average (TWA)	Registrant study that collected air measurements in 7 homes from 7 days to 1 year post-treatment.	13,000 (2,100-30,000)	3800 (600-8700)	Median MOE with range of MOEs presented in parentheses. Values adjusted from 1% ai (typical rate) to 0.5% ai (minimum rate). Assumes a child spend 20 hours in a treated residence.
1-Year Incremental TWA		3,800 (930-8,800)	1,100 (270-2,500)	
Crawl-Space-type Construction				
90-Day Incremental Time-weighted- average (TWA)	See comments under basement construction.	7,300 (3,300-25,000)	2,100 (950-7,200)	See comments under basement construction.
1-Year Incremental TWA		1,800 (1,200-7,400)	530 (340-2,100)	
Slab Type Construction				
90-Day Incremental Time-weighted- average (TWA)	See comments under basement construction.	6,600 (1,500-20,000)	1,900 (440-5,800)	See comments under basement construction.
1-Year Incremental TWA		2,100 (960-7,600)	600 (280-2,200)	
Plenum-Type Construction				
90-Day Incremental Time-weighted- average (TWA)	See comments under basement construction.	6,600 (1,600 - 22,000)	1,900 (460 - 6,400)	See comments under basement construction. 1-Year incremental TWA based on five houses, due to insufficient sampling for two houses. Sampling not conducted beyond days 30 and 7 for houses P-6 and P-7, respectively. Based on available data, these houses had higher air concentrations than the other houses.
1-Year Incremental TWA		2,600 (940-9,500)	760 (270-2,700)	

Table 15. Estimates of Post-Application Risks to Residents/Recreational Users				
Reentry Scenario	**Method of Evaluation**	**Central-tendency MOE**		**Risk Characterization/ Uncertainties**
		Adult	**Child**	
(5) Insecticidal Dust Products (Insufficient data to evaluate; see text)				
Broadcast Turf Application (Residential/Recreational) (Short-term)				
(6) Chlorpyrifos Spray (Dursban Turf Insecticide)				
Inhalation	Biomonitoring Study, with environmental measurements. Application of 0.29% chlorpyrifos spray at 4 lb ai/acre	170	20	Average represents central-tendency risk estimates based on arithmetic mean exposure from biomonitoring study in adults, where chlorpyrifos applied at the maximum label rate of 4 lb ai/acre. Based on 2 hour dermal contact with lawn the day of treatment. Maximum represents the highest exposed individual in the study. Study does not adequately address frequent hand to mouth activity of children, or incidental ingestion of soil or residues on treated grass by children. Application at typical rate of 1 lb ai/acre would potentially result in lower exposures (see below).
Dermal		10	12	
Oral		NE	400	
Total Absorbed Dose		Average: 9 -24 Maximum: 5.6-15	Average: 7.5-15 Maximum: 6-12	
Total Absorbed Dose	Biomonitoring Study with adjustment for 1 lb ai/acre	Average: 36-96	Average: 30-60	Low to Central-tendency risk estimates, based on typical application rate of 1 lb ai/acre.
(7) Granular Formulation of 0.5% Chlorpyrifos (Dursban Insecticide) (1.8 lb ai/acre)				
Inhalation	Biomonitoring Study, with environmental measurements	330	400	Average represents central-tendency risk estimates based on arithmetic mean exposure from biomonitoring study in adults. Based on 2 hour dermal contact with lawn the day of treatment; does not adequately address frequent hand to mouth activity of children, or incidental ingestion of soil or granules by children. Maximum MOE is for the highest exposed individual in the study.
Dermal		190	90	
Oral		NE	6000	
Total Absorbed Dose		Average: 110-120 Maximum: 42-45	Average: 73-75 Maximum: 29	
(8) Golf Course Treatment (Dursban Turf Insecticide; EPA Reg 62719-35) (1-4 lb ai/acre) (Short-term)				
Adolescent Golfer (12 yrs; 44kg)	Residential SOPs and surrogate residue data from flurprimidol study the day of treatment	360 (4 lb ai/acre) 1500 (1 lb ai/acre)		High-end risk estimates. Assumes exclusively dermal exposure the day of turf treatment Assumes a 4 hour exposure for a 18 hole round of golf.

Table 15. Estimates of Post-Application Risks to Residents/Recreational Users				
Reentry Scenario	Method of Evaluation	Central-tendency MOE		Risk Characterization/ Uncertainties
		Adult	Child	
Adult Golfer		600 (4 lb ai/acre) 2400 (1 lb ai/acre)		

Table 15. Estimates of Post-Application Risks to Residents/Recreational Users				
Reentry Scenario	**Method of Evaluation**	**Central-tendency MOE**		**Risk Characterization/ Uncertainties**
		Adult	**Child**	
(9) Aerial and Ground-Based Fogger Mosquitocide Application (Mosquitomist One, EPA Reg. 8329-24) (0.01 lb ai/acre) (Short-term)				
Dermal	Literature studies, the AgDrift Model and the updated Residential SOPs	42,000	26,000	High-end risk estimates based on the updated Residential SOPs. Assumes long-term inhalation exposure is negligible based on low application rate and infinite dilution.
Oral (hand to mouth)		NE	13,000	
Oral (Turfgrass Ingestion)		NE	54,000	
Oral (Soil Ingestion)		NE	20,000,000	
Total Exposure		42,000	15,000	
(10) Yard and Ornamental Sprays (Evaluated based on analogy to Lawn Products; see text)				
(11) Perimeter Treatment of Residence (Dursban Pro, EPA Reg. 62719-166) (4.35 lb ai/acre) (Short-term)				
Dermal	Updated Residential SOPs Residential	NE	8 minutes of play is equivalent to a MOE of 1000	High-end risk estimates based on the updated Residential SOPs. Assumes a child plays on treated turf the day of treatment. The most critical items are the probability that a child would play within 6 to 10 feet of a residence and for what duration a child would be in the treatment zone.
Oral (hand to mouth)		NE	7 hand to mouth events is equivalent to a MOE of 1000	
Oral (Soil Ingestion)		NE	MOE = 2300	

4.4.4.4 Incident Reports

Chlorpyrifos is one of the most widely used insecticides in the home both by consumers and PCOs or exterminators. In a 1990 EPA-sponsored survey of pesticide use in households, chlorpyrifos was the fourth most commonly used insecticide, present in 18% of all households. A 1993 EPA survey of PCOs found it was the number one insecticide in use and accounted for a quarter of the poundage used in residential settings. Consequently, there have been many reports of human exposure and poisonings due to the widespread use of chlorpyrifos. The human poisoning incidents associated with chlorpyrifos exposure have been evaluated and summarized in the attached memorandum from J. Blondell to D. Smegal, April 20, 2000. HED notes that approximately 98% of chlorpyrifos exposures discussed below are due to products removed under the risk mitigation plan.

Data from the Nation's Poison Control Centers in 1996 reported approximately 116,000 unintentional exposures to all pesticides, of which, 16% were due to organophosphate (OP) pesticides, and 5,188 or 4.5% were attributed to chlorpyrifos. These numbers are based on exposures to single products, a small proportion of which may contain additional active ingredients besides chlorpyrifos. Given that 30% of the organophosphate poisonings were not specifically identified by active ingredient, the actual number of chlorpyrifos cases is probably close to 7,000 or 6% of all pesticide-related exposures. Many of these exposures involve small children who were exposed but never developed symptoms. In 1996 there were 1,109 symptomatic cases related to chlorpyrifos that were judged to have effects related to the exposure, although most (83%) had only minor symptoms (e.g., headache, nausea, vomiting, dizziness and diarrhea) that could be treated at home. From 1993 through 1996, there were an average of 116 unintentional chlorpyrifos cases per year with moderate to severe outcomes (including one fatality) reported in residential settings.

The possibility of risk from chlorpyrifos exposure is very similar to the other OP pesticides (e.g., diazinon, malathion, dichlorvos) that have significant residential uses for both children and adults. The one exception is the percent of cases with fatal or life-threatening outcome (not including suicide attempts), where chlorpyrifos had the highest percentage (0.46% based on 18 cases) of any of the other 13 OP pesticides, that was 50% higher than any of the non-OP pesticides. Between 1993 and 1996, there was one fatality and 34 life-threatening cases attributed to chlorpyrifos exposure. The fatality was a 22 month old boy who accidently ingested chlorpyrifos that had

been placed in a cup. Measures called for in the 1997 Chlorpyrifos Risk Reduction Plan, in part, were aimed a preventing such poisoning incidents.

Chlorpyrifos ranked third of the 13 OPs for serious outcomes resulting from exposure to environmental residues left after application or use. Environmental residues accounted for 15% of the chlorpyrifos exposures and 30% of the cases with serious outcomes (moderate or life-threatening), which was double the incidence for non-OP pesticides.

A particular concern with chlorpyrifos are reports of exposures and poisonings related to use by PCOs. A review of the Poison Control Center data for four years (1993-1996) found over 1000 reports of exposure (250 per year) to chlorpyrifos products that would most commonly be used by PCOs in residential settings. A total of 325 of these cases were symptomatic, 241 cases were seen in a health care facility, 35 were hospitalized and 16 were admitted to an intensive care unit (ICU). Chlorpyrifos PCO products accounted for 9% of the exposures, but 21-24% of the life-threatening/fata cases, hospitalized cases and cases seen in an ICU. Note that the number of cases involving PCO products is relatively small compared to the exposure and symptomatic cases involving consumer products. Just 4% of the product-identified chlorpyrifos exposures in children under age six involved PCO products, and for adults and children over age six the figure was 15%. Also, some of the more serious cases, both for PCO and homeowner products, were due to broadcast carpet treatment, fogger and pet uses that were voluntarily canceled in 1997.

Another source of concern with all the OP pesticides, including chlorpyrifos, are the frequent anecdotal reports of chronic neurobehavioral effects and multiple chemical sensitivity. Kilburn (1999) documented neurobehavioral effects (including signs consistent with peripheral neuropathy in 11 cases) among 22 patients reporting exposure to chlorpyrifos, 10 of which were self-referred and 12 referred by attorneys. In addition to these reports, there were 14 self-reported but unconfirmed cases (without medical documentation) of chronic neurobehavioral effects submitted by Dow AgroSciences during 1998-1999. Another 73 cases were reported to EPA during the public comment period (October-December 1999) for chlorpyrifos. A few of these cases may have overlapped the reports from Kilburn and Dow AgroSciences. Twelve of the 73 cases provided some, often very limited, medical documentation of their effects. Out of all of the cases reported by Kilburn, Dow AgroSciences or directly to EPA there were only about 3-4 with laboratory confirmation (e.g., reduced cholinesterase) of their

exposures. Neurobehavioral effects reported include persistent headaches, blurred vision, muscle weakness, fatigue, and problems with mental function including memory, concentration, depression, and irritability.

HED suspects that these chronic neurobehavioral effects are caused by the acute poisoning, partly from a case-control study in California partly from case-control (cross sectional) studies of other OP pesticides similar to chlorpyrifos, and most recently from a NIOSH study. With EPA support, NIOSH completed a study of 191 current and former PCOs that apply chlorpyrifos as a termiticide in North Carolina. An extensive battery of neurological and neurobehavioral tests was administered. The study (Steenland et al. 2000), concluded "this cross-sectional study of workers exposed to chlorpyrifos . . . found few exposure related effects for most tests, including a clinical exam. However, the exposed did not perform as well as the non-exposed on pegboard turning tests and some postural sway tests. Furthermore, exposed subjects reported more symptoms than non-exposed subjects; this is a cause for concern because previous studies lend some support to this finding." Among acutely poisoned subjects the study stated, "Eight men who reported past chlorpyrifos poisoning had a pattern of low performance on a number of tests, which is consistent with prior reports of chronic effects of organophosphate poisoning." Finally, the study noted the following reservation, partly due to the relatively heavy exposure experienced by study participants, "Although this was a relatively large study based on a well-defined target population, the workers we studied may not be representative of all exposed workers and caution should be exercised in generalizing our results." (Steenland et al. 2000). These findings are consistent with an earlier review that suggested chlorpyrifos may be a cause of chronic neurobehavioral effects in some subsets of sensitive people who have been poisoned (Blondell and Dobozy 1997). In addition to the studies described above, DAS has agreed to undertake an epidemiologic study of manufacturing workers.

As noted previously, four uses of chlorpyrifos have been voluntarily canceled and removed from the market: paint additives; shampoos, sprays and dips used on pets; indoor broadcast flea control products; and household foggers. Poison Control Center data for 1993-1996 suggest that as many as 20-25% of symptomatic exposures in residential settings were related to these uses. All of these residential uses involve either concentrates or widespread applications that involve greater potential for exposure to consumers than do other forms and uses of chlorpyrifos. Therefore, substantially less exposures and hazards are expected when additional years of

poisoning surveillance data become available. DAS is continuing its' efforts to monitor poisoning incidents through its agreement with a Poison Control Center that takes telephone contacts from the public and the health care community concerning chlorpyrifos. Follow up information to determine the circumstances that lead to exposure and poisoning should be useful.

4.4.5 Pet Incident Reports

A review and analysis of the poisoning incident reports on domestic animals for chlorpyrifos was conducted in 1995 (attached memo from V. Dobozy to B. Kitchens, January 23, 1995) and was updated in 1999 (attached memo from V. Dobozy to D. Smegal, April 26, 1999, D255514). In the 1995 analysis, poisoning incidents in dogs and cats were categorized as exposure by direct applications (flea and tick dips, sprays, collars, etc) or by premise applications (household and lawn treatments). The analysis found that the majority of the incidents in domestic animals involved cats, although the chemical is registered only for use in flea collars for this species. Cats that were exposed to products registered only for use on dogs, mainly dips, experienced a high incidence of death (30%). There was also evidence of misuse of treatment products, including practices such as applying these products directly to animals and not removing pets from premises during applications.

In 1996, PR Notice 96-6 was finalized, which requires the revision of labels for all products administered directly to animals to ensure adequate directions for use and warning information. In 1997, the registrant voluntarily agreed to cancel chlorpyrifos registrations for indoor broadcast flea control and direct application pet products (sprays, shampoos, and dips), except flea collars, to establish specific protection measures for pets during and immediately after application, and to expedite implementation of PR Notice 96-6 on pet products.

An evaluation of incident reports for domestic animals for the years 1996 through 1998 (memo from V. Dobozy to D. Smegal, April 26, 1999, D255514) revealed that there has been a decrease in the percentage of incidents resulting from exposure to products registered for direct use on animals, but an increase in the percentage of incidents resulting from premise exposure. In addition, deaths are still being reported, especially for cats. The cancellation of indoor broadcast flea control applications and products for direct application to dogs and cats should reduce the risk of serious adverse reactions and deaths, however time is required to eliminate all chlorpyrifos products from store shelves. Therefore, it may be premature to review the Incident Data System (IDS) for evidence that these actions were effective.

4.5 Chlorpyrifos Exposure Estimates in the U.S. Population

Because of chlorpyrifos' extensive use on food and in homes and the workplace, the majority of the U.S. population is exposed to this pesticide. Literature studies, in addition to several of the registrant-submitted biomonitoring studies, have estimated typical or baseline exposure to chlorpyrifos by measuring the urinary excretion of 3,5,6-TCP, the primary metabolite of chlorpyrifos. TCP has a biological half-life of approximately 27 hours, therefore, the urinary TCP levels reflect recent exposure. It should be noted however, that exposure to chlorpyrifos-methyl, 3,5,6-TCP (the animal, and plant metabolite and environmental degradate of chlorpyrifos and chlorpyrifos-methyl), and trichlorpyr (a herbicide) also contribute to an unknown degree to 3,5,6-TCP urinary concentrations, thus the chlorpyrifos exposure estimates presented in this section represent an upper-bound estimate. Chlorpyrifos contributes significantly more to urinary TCP than chlorpyrifos-methyl and trichlorpyr based on relative annual U.S. usage of approximately 21 to 24 million pounds of chlorpyrifos (of which approximately 11 million are used in residential and recreational settings) versus 92,000 pounds of chlorpyrifos-methyl and 700,000 pounds of trichlorpyr.

HED has conducted a preliminary risk assessment for TCP, which is in the attached memorandum from S. Knizner to D. Smegal, D265035 June 5, 2000.

Table 16 summarizes the typical upper-bound baseline exposure to chlorpyrifos estimated from the registrant submitted biomonitoring studies of TCP measurements, and the scientific literature. These values represent worst case estimates because all of the TCP was attributed to chlorpyrifos.

Registrant Residential Biomonitoring Studies

DAS recently conducted four biomonitoring studies to quantify exposures to residential populations following the use of chlorpyrifos products in the home. Volunteers were typically adults of both sexes between the ages of 25 and 65. Other details were not provided (i.e., ethnicity). For all of these studies, baseline chlorpyrifos exposures of the volunteers were quantified by analysis of urinary 3,5,6-TCP prior to commencement of the study. Quantification of baseline chlorpyrifos exposure for each volunteer was necessary in order to determine actual exposure associated with a product's use. For each of these studies, baseline TCP measurements were subtracted from total TCP measurements to quantify chlorpyrifos exposure in the biomonitoring study. In addition, residents were instructed to avoid chlorpyrifos exposure for several days (typically one week to 10 days) prior to the measurement of baseline levels. Therefore, the baseline exposures are most likely attributed to dietary exposure of chlorpyrifos, chlorpyrifos-methyl and TCP.

In August 1999, DAS submitted a TCP Biomonitoring study that assesses children's potential household exposure to chlorpyrifos and its environmental degradate, TCP (MRID 44889501). The study evaluated urinary TCP concentrations of 416 children 0-6 years of age in North and South Carolina; 120 children were from households treated with a termiticide containing chlorpyrifos, and 296 children were from households identified from the general population sample. TCP was detected in 100% of the children's urine. The 24 hour TCP excretion ranged from 0.09 to 75.79 Fg TCP/g creatinine/kg body weight, with a mean value of 1.19 Fg TCP/g creatinine/kg body weight. These values correlate to approximately 0.045 to 38 Fg chlorpyrifos /kg/day, with a mean value of 0.6 Fg/kg/day. It should be noted that 73% (303/413) and 11% (47/413) of the children in this survey lived in homes that had been treated with a chlorpyrifos-containing insecticide indoors or with a termiticide, respectively within the past year. In addition, 64% of the children (264/412) also were from homes that had a lawn treatment within the past year. HED is currently reviewing this study.

Scientific Literature

The study published by Hill et al. (1995) measured the biomarker 3,5,6-TCP in 993 adults (20-59 years old) participating in the National Health and Nutrition Examination Survey III, known as NHANES III from 1988 - 1994. The individuals were selected from a broad spectrum of the U.S. population reflecting both sexes and different age groups, races/ethnicities, urban/rural residences and regions of the country. 3,5,6-TCP was detected in 82% of the individuals evaluated. The average TCP concentration was 4.5 Fg/L or 3.1 Fg TCP/g creatinine. The results of NHANES III differ significantly from the NHANES II survey collected between 1976 and 1980, where only 5.8% of the 6990 people evaluated had concentrations of 3,5,6-TCP greater than the detection limit of 5 Fg/L. In the NHANES III survey, 31% of the 993 people had 3,5,6-TCP concentrations greater than 5 Fg/L. It should be noted however, that the lower detection limit of 1 Fg/L in the NHANES III study could partially account for the increased frequency of detection of 82%. The results of this study are presented below in Table 14. It is possible that the registration of chlorpyrifos-methyl for use on stored grains in 1985 contributes to the increased frequency and concentration of TCP measurements between the NHANES II and III results. In addition, chlorpyrifos-methyl was detected at greater frequencies than chlorpyrifos in the 1991-1997 Total Diet Study (FDA 1999). In this study,100% of samples for several commodities containing flour (i.e., whole wheat bread, tortilla flour, rye bread, cracked wheat bread, english muffin, teething biscuits, pretzels, fish sticks, white roll, and butter type crackers) contained measurable chlorpyrifos-methyl residues.

A recent study of 65 recently-exposed termiticide applicators (Steenland et al. 2000) reported an average urinary TCP level of 629.5 Fg/L, compared to the 4.5 Fg/L for the general U.S. population from Hill et al. (1995).

The Minnesota Children's Pesticide Exposure Study, which is one of the National Human Exposure Assessment Surveys (NHEXAS), evaluated 102 children ages 3-12 (mean 7.6 ± 2.9 yrs), stratified by those with more frequent residential insecticide usage (personal communication with James Quackenboss, March 1, 1999). This study was initiated to assess children's actual exposures to pesticides. The study examined the relationship between environmental concentrations and urinary biomarker levels of 3,5,6-TCP from a population-based study of total exposure in urban and non-urban children. Tap water, personal, indoor, and outdoor air, house dust, and soil were monitored over 6 days while food and beverage monitoring was conducted over 4 days. Urine samples were obtained for 87% (89) of the study subjects. Preliminary data were presented at the International Society for Environmental Epidemiology (ISEA) conference in Boston in August 1998 (Adgate et al. 1998), where 92% of the 89 children had measurable levels of 3,5,6-TCP in their urine. It should be noted, however, that the study over sampled homes that frequently used pesticides, and 30% of the households had used chlorpyrifos. The results from the metabolite analysis suggest that these children have higher concentrations of 3,5,6-TCP than was reported for the NHANES-III adult population (medians of 8 and 2 Fg/L TCP, respectively) (Quackenboss et al. 1998). The final study results are anticipated to be available in 2000.

Macintosh et al. (1999) evaluated urinary TCP levels in 80 individuals in Maryland during 1995-1996. Up to six samples were collected from each individual over a period of a year. TCP was detected in 96% of the 346 samples at a median concentration of 5.3 Fg/L and 4.6 Fg/g creatinine. The geometric mean concentrations of TCP were significantly greater in samples collected during the spring and summer of 1996 than in the preceding fall and winter. In addition, the geometric mean TCP concentrations differed significantly between Caucasian (GM = 5.7 Fg/g creatinine) and African-American (GM = 4 Fg/ g creatinine) participants and among education levels but were not significantly different among groups classified by gender, age, or household income. The mean and median TCP concentrations in this study (5.8 and 4.6 Fg/g creatinine) are approximately twofold greater than those measured in the NHANES III (3.1 and 2.2 Fg/g creatinine, respectively) (Hill et al. 1995), however the upper end of the distributions are approximately equal. Individual urinary TCP levels varied over time and were highly variable, indicating that a single measure of urinary TCP levels is not sufficient to adequately characterize the relative magnitude of a person's typical exposure to chlorpyrifos.

Buckley et al. (1997) evaluated 18 nonsmoking adults from nine homes in the Lower Rio Grande Valley (LRGV) in Texas during the spring and summer 1993. Urinary TCP was significantly higher in the summer relative to the spring, and was correlated with air and dust concentrations. TCP was detected in 77% (13/17) and 92% (11/12) of the spring and summer samples, respectively at median concentrations of 1.9 and 3.2 Fg/L, respectively.

Table 16 summarizes the typical upper-bound baseline exposure to chlorpyrifos estimated from the Hill et al. (1995) and DAS biomonitoring studies of TCP measurements. These values represent worst case estimates because all of the TCP was attributed to chlorpyrifos. All exposure estimates have been normalized for creatinine excretion. The assumptions and equations are presented in the footnotes.

Table 16
Upper Bound Chlorpyrifos Exposure Estimates Based on Biomonitoring of Urinary TCP

Source/Study	Sample Size	Percent with TCP in urine	Mean Chlorpyrifos Dose Fg/kg/day	95th Percentile Fg/kg/day	Range of Chlorpyrifos Dose Fg/kg/day
Residential Biomonitoring Studies					
Child TCP Biomonitoring study (0-6 yrs old, North and South Carolina, 1998) (a)	416	100%	0.6	1.32	0.045-4.7
Residential exposures from Lawn treated with Chlorpyrifos Spray (MRID 43013501) (Adults) (b)	8	100%	0.3	NE	0.09 - 0.6
Residential Exposures from Lawn treated with Granular Chlorpyrifos (MRID 44167101) (Adults) (b)	9	100%	0.5	NE	0.21 - 1.47
Residential Exposure from Crack and Crevice Application (MRID 44458201) (Adults) (b)	6	100%	0.4	NE	0.1-0.86
Residential Exposures from Application of a Ready-to-Use Formulated Product (MRID 44739301) (Adults) (b)	15	100%	0.12	NE	0.05-0.3
Literature Studies					
Hill et al. 1995 (NHANES III) (Adults, 1988-1994) (c)	993	82%	0.2 (b)	0.52	ND - 2
MacIntosh et al. 1999 (Adults, Maryland, 1995-1996) (d)	80 people (329 samples)	96%	0.37	1	0.013-2.2
Buckley et al. (1997) (Adults, Texas, 1993) (e)	18	Spring: 77% Summer: 92%			

ND = not detected

NE = not estimated

(a) Creatinine adjusted concentrations for 24 hour TCP excretion ranged from 0.09 to 15.8 Fg TCP/g creatinine/kg body weight, with a mean value of 1.19 Fg TCP/g creatinine/kg. In the initial study, the highest child was 75.79 Fg TCP/g creatinine/kg, which is equal to approximately 38 Fg/kg/day chlorpyrifos. A more recent submission, March 2000, reported lower levels of TCP in this child of 15.8 Fg TCP/g creatinine/kg, which is equivalent to approximately 4.7 Fg/kg/day chlorpyrifos. The 95th percentile was 2.63 Fg TCP/g creatinine/kg. Assumes child specific body weight, and average creatinine excretion of 0.2 g/day from 416 children. Assumes steady-state between exposure and excretion.

(b) Based on pre-study 3,5,6-TCP results in urine. See HED study reviews for details

(c) Creatinine adjusted concentrations of mean 3.1 and maximum of 34 Fg TCP/g creatinine, respectively that assumes an average creatinine excretion rate of 1.8 g/day (Tietz 1982), a body weight of 70 kg, and that 72% of chlorpyrifos is excreted in the urine. A molecular weight adjustment was also made 350.6 chlorpyrifos/ 198 TCP. Assumes steady-state between exposure and excretion. Example calculation: Dose (Fg/kg/day) = [(3.1 Fg TCP/g creatinine * 350.6/198 * 1.8 g/day) / (70 kg * 0.72 (fraction chlorpyrifos excreted as TCP)].

(d) creatinine adjusted concentrations of <0.2, 5.8, 16 and 35 Fg TCP/g creatinine for minimum, mean, 95th percentile and maximum, respectively. Assumes an average creatinine excretion rate of 1.8 g/day (Tietz 1982), a body weight of 70 kg, and that 72% of chlorpyrifos is excreted in the urine. A molecular weight adjustment was also made 350.6 chlorpyrifos/ 198 TCP. Example calculation: Dose (Fg/kg/day) = [(35 Fg TCP/g creatinine * 350.6/198 * 1.8 g/day) / (70 kg * 0.72 (fraction chlorpyrifos excreted as TCP)].

(e) Creatinine adjusted concentrations not presented. Median TCP concentrations of 1.9 and 3.2 Fg/L and maximum concentrations of 6.4 and 11 Fg/L for spring and summer, respectively.

5.0 Aggregate Risk Assessments and Risk Characterization

The Food Quality Protection Act amendments to the Federal Food, Drug, and Cosmetic Act (FFDCA, Section 408(b)(2)(A)(ii)) require that for establishing a pesticide tolerance "that there is reasonable certainty that no harm will result from aggregate exposure to pesticide chemical residue, including all anticipated dietary exposures and other exposures for which there are reliable information." Aggregate exposure is the total exposure to a single chemical (or its residues) that may occur from dietary (i.e., food, and drinking water), residential and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation). Aggregate risk assessments are typically conducted for acute (1 day), short-term (1-30 days), intermediate-term (30 days to several months), and chronic (several months to lifetime) exposure.

DAS has submitted a probabilistic Integrated Exposure Assessment (MRID No. 44104001, September 1996). This submission is in internal HED review, because the Agency policy on aggregate probabilistic risk assessment is still in development. This submission, however, has been used by the Agency in developing policy and will be evaluated once this policy is finalized and has undergone peer review.

The total residential MOEs (dermal, inhalation, and inadvertent oral exposures) for all the residential post-application exposure scenarios, except mosquitocide use, and golf course use alone exceed HED's level of concern. In addition the acute dietary exposure and risk estimates exceed HED's level of concern. However, HED conducted acute, short-term and chronic aggregate assessments assuming the mitigation plan is adopted. As noted previously, the mitigation plan would reduce potential chlorpyrifos exposures on apples, grapes and tomatoes, and mitigate the residential/recreational exposures.

5.1 Acute Aggregate Risk

The acute aggregate risk estimate to chlorpyrifos addresses exposures from food and drinking water. For the highly refined acute probabilistic dietary exposure analysis, PDP, FDA and NFS monitoring data were used to the greatest extent possible, along with field trial data, and cooking and processing factors to assess dietary exposures. This aggregate assessment incorporates the mitigation plan (i.e., reduction of apple tolerance to 0.01 ppm based on dormant application, reduction of grape tolerance to 0.01 ppm based on domestic use pattern and deletion of the use on tomatoes).

With the mitigation measures, the chlorpyrifos acute dietary risk estimates range from 4.1% to 82% of the aPAD, with children (1-6 yrs) being the highest exposed population subgroup. Thus, the mitigated acute dietary (food) risk estimate associated with chlorpyrifos exposure is below the Agency's level of concern. Using conservative screening-level models, the acute estimated concentrations (EECs) of chlorpyrifos in groundwater (SCI-GROW) range from 0.007 to 0.103 Fg/L. The acute surface water EECs, based on upper-bound

monitoring data results, are 0.026 to 0.4 Fg/L, respectively. As shown previously on Table 7, and on Table 17 below, the EECs are less than the DWLOCs for all populations (highest EEC of 0.4 Fg/L is less than the lowest DWLOC of 0.9 Fg/L), indicating that acute food and drinking water exposures (except possible well contamination) do not exceed HED's level of concern. It should be noted that neither the SCI-GROW model nor the monitoring data reflect concentrations after dilution (from source to treatment to tap) or drinking water treatment. HED concludes that **acute aggregate chlorpyrifos exposure in food and water does not exceed HED's level of concern.**

Table 17
Summary of Acute Aggregate Exposure
Includes Risk Mitigation

Population Subgroup (a)	Acute PAD (Fg/kg/day)	Food Exposure 99.9th (Fg/kg/day) (b)	Max. Water Exposure (Fg/kg/day) (c)	Surface Water (Monitoring Data) (Fg/L)	Ground Water SCI-GROW, (excluding well contamination) (Fg/L)	Acute DWLOC (Fg/L) (d,e,f)
U.S. Population	5	0.237	4.76	0.026 to 0.4	0.007 to 0.103	166
All Infants (< 1 Year)	0.5	0.258	0.242			2.4
Children (1-6 years)	0.5	0.410	0.09			0.9
Females (13-50 years)	0.5	0.201	0.299			9

(a) In addition to the U.S. population (all seasons), the most highly exposed subgroup within each of the infants, children, female groups is listed.
(b) 99.9th percentile exposure. Values are from Table 3 (and rounded).
(c) Maximum Water Exposure (Fg/kg/day) = Acute PAD (Fg/kg/day) - [Acute Food Exposure (Fg/kg/day)].
(d) DWLOC (Fg/L) = Maximum water exposure (Fg/kg/day) x body wt (kg) ÷ water consumed daily (L/day)]
(e) HED default body weights are: general U.S. population, 70 kg; adult females, 60 kg; and infants/children, 10 kg.
(f) HED default daily drinking water rates are 2 L/day for adults and 1 L/day for children.

Acute exposure to chlorpyrifos in groundwater as a result of well contamination from termiticide use could potentially result in exposures of concern. However, as noted previously, the groundwater exposures from well contamination resulting from termiticide use are highly localized. The implementation of PR 96-7 for termiticides has reduced the reported incidents of groundwater contamination resulting from termiticide treatments. For example, incidents associated with termiticide use were 28.2 per 100,000 homes in 1997 (pre PR-96-7), and were 8.3

per 100,000 homes in 1998 (post PR-96-7).

5.2 Short-Term Aggregate Risk

The short-term aggregate risk estimate includes chronic dietary (food and water) from chlorpyrifos uses, and short-term non-occupational exposures (i.e., residential/recreational uses). As noted previously, this aggregate assessment is based on the mitigation plan that would reduce potential chlorpyrifos exposures in food (apples, grapes and tomatoes) and in the residential/recreational environment. This assessment evaluates potential exposures resulting from continued chlorpyrifos use on golf courses at a reduced rate of 1 lb ai/acre (i.e., risks to golfers), in addition to potential exposures as a result of mosquito abatement activities.

Table 18 presents the aggregate exposure estimates for chlorpyrifos from diet and residential/non-occupational uses (golfing and mosquitocide abatement activities). Based on the mitigation plan, it was assumed that children (1-6 years) could be exposed to chlorpyrifos residues on turf as a result of ground-based fogger applications of a chlorpyrifos-containing mosquitocide, and through dietary exposures. Children 7-12 years were assumed to be dermally exposed to chlorpyrifos residues while playing golf (the day of treatment), and to ingest chlorpyrifos residues in the diet. Female residents were assumed to be concurrently exposed to chlorpyrifos via mosquito abatement activities (i.e., dermal contact with residues on turf), golfing (dermal contact turf residues the day of treatment), in addition through dietary exposures. The results of the exposure analysis for the individual scenarios are presented in detail in the Occupational /Residential Exposure Chapter for the RED for Chlorpyrifos (D266562, June 2000).

As shown on Table 18, aggregate MOEs are greater than 1000 for children 1-6 years, children 7-12 years and females 13-50 years, and therefore do not exceed HED's level of concern. Therefore, short-term DWLOCs were estimated to account for potential drinking water exposures.

Table 18
Summary of Aggregate Short-Term Exposure
Chronic Diet and Short-Term Residential Use
(Excludes Water)
Includes Risk Mitigation

Population Subgroup	Dietary Exposure with Risk Mitigation	Short-Term Residential/Recreational Exposure (Fg/kg/day)/ MOE Risk Mitigation			Total Aggregate MOE Estimate (b)
		Mosquitocide Postapplication		Golf Course Postapplication Exposure (1 lb ai/acre)	Diet and Residential/ Recreational Exposure
	Chronic Diet Exposure with FHE (Fg/kg BW/day) (a)/ MOE	Oral	Dermal	Dermal	Oral and Dermal
Children (1-6 years)	0.008 MOE = 62,500	0.013 MOE = 38,500	0.19 MOE = 26,000	NE	12,000
Children (7-12 years)	0.015 MOE = 33,000	NE	NE	3.4 MOE = 1,500	1,400
Females 13-50	0.006 MOE = 83,000	NE	0.14 (c) MOE= 36,000	2.45 (c) MOE = 2,000	1,900

NE = not evaluated.
FHE = Food Handling Establishment Use

(a) MOE calculated based on acute oral NOAEL of 500 Fg/kg/day, and short-term dermal NOAEL of 5000 Fg/kg/day for dermal exposures. No dermal absorption is necessary because dermal NOAEL is based on a dermal rat study.

(b) Oral and dermal exposures were combined because the oral and dermal endpoints are both based on plasma and RBC ChE inhibition.

(c) Adjusted from 70 kg to 60 kg for aggregate exposure.

The short-term DWLOC values are presented in Table 19. For each population subgroup listed, the acute PAD and the chronic dietary (food) exposure (from Table 4) for that subgroup were used to calculate the short-term DWLOC for the subgroup, using the formulas in footnotes of Table 19. The EECs are less than the DWLOCs for all populations (highest EEC of 0.1 Fg/L is less than the lowest DWLOC of 1.4 Fg/L), indicating that chronic food and drinking water exposures (except possible well contamination), in addition to exposures from mosquitocide abatement and golfing activities do not exceed HED's level of concern. In conclusion, potential **short-term aggregate exposure to chlorpyrifos resulting from food, water and residential/recreational use, assuming the mitigation plan is adopted, does not exceed HED's level of concern.** This analysis is considered conservative because, HED assumed that there could be concurrent residential and recreational exposures to chlorpyrifos (i.e., golfing and mosquitocide abatement activities on the same day). In addition, neither the SCI-GROW model nor the monitoring data reflect concentrations after dilution (from source to treatment to tap) or drinking water treatment.

Table 19
Summary of Short-Term Aggregate Exposure DWLOCs
Chronic Diet and Short-Term Residential Use
Includes Risk Mitigation

Population Subgroup (a)	Acute oral NOAEL (Fg/kg/ day)	Short-Term MOE (Food and Residential) (Fg/kg/day) (a)	MOE Water (b)	Max. Water Exposure (Fg/kg/ day) c)	Surface Water (Monitoring Data) (Fg/L)	Ground Water SCI-GROW, (excluding well contamination) (Fg/L)	Short-Term DWLOC (Fg/L) (d,e,f)
Children (1-6 years)	500	1,200	1,090	0.4587	0.026	0.007 to 0.103	4.5
Children (7-12 years)		1,400	3,450	0.14			1.4
Females (13-50 years)		1,900	2,100	0.238			7.1

(a) Values are from Table 18.
(b) MOE_{WATER} = 1 / [(1/MOE_{AGG} - [1/MOE_{FOOD} + 1/MOE_{DERMAL} + 1/MOE_{ORAL}]), where MOE_{AGG} is 1000.
(c) Maximum Water Exposure (Fg/kg/day) = Acute NOAEL of 500 (Fg/kg/day)÷ MOE_{WATER}
(d) DWLOC (Fg/L) = Maximum water exposure (Fg/kg/day) x body wt (kg) ÷ water consumed daily (L/day)]
(e) HED default body weights are: adult females, 60 kg; and infants/children, 10 kg.
(f) HED default daily drinking water rates are 2 L/day for adults and 1 L/day for children.

5.3 Intermediate-Term Aggregate Risk

Based on the mitigation plan, there are no residential/recreational uses that result in exclusively intermediate-term exposures (i.e., > 30 days but less than 6 months). Therefore, an intermediate-term aggregate risk estimate was not evaluated.

5.4 Chronic Aggregate Risk

The chronic aggregate risk estimate to chlorpyrifos addresses exposures from food and drinking water. For the highly refined chronic dietary exposure analysis, PDP, FDA and NFS monitoring data were used to the greatest extent possible, along with field trial data, and cooking and processing factors to assess dietary exposures. This aggregate assessment incorporates the mitigation plan (i.e., reduction of apple tolerance to 0.01 ppm based on dormant application, reduction of grape tolerance to 0.01 ppm based on domestic use pattern and deletion of the use on tomatoes), and assumes there are no chronic exposures from termiticide treatments.

The chlorpyrifos chronic noncancer dietary risk estimates range from 2.5 to 51% of the cPAD, with children (1-6 yrs) being the highest exposed population subgroup. Thus, the chronic dietary (food) risk estimate associated with chlorpyrifos exposure is below the Agency's level of concern.

Using conservative screening-level models the groundwater EECs range from 0.007 to 0.103 Fg/L. The upper-bound surface water EEC, based on monitoring data, is 0.026 Fg/L. As noted previously, DWLOCs were calculated based on food (including food handling establishment uses) and water exposure alone to account for the mitigation options. The chronic non-cancer DWLOC values were presented previously in Table 8, and are shown below on Table 20. For each population subgroup listed, the chronic PAD and the chronic dietary (food) exposure (from Table 4) for that subgroup were used to calculate the chronic DWLOC for the subgroup, using the formulas in footnotes of Table 20. As shown, the upper-bound EEC of 0.103 Fg/L is less than the DWLOCs, and therefore does not exceed HED's level of concern. It should be noted that neither the SCIGROW model nor the monitoring data reflect actual drinking water concentrations after dilution (from source to tap) or drinking water treatment.

Table 20
Summary of Short-Term Aggregate Exposure DWLOCs
Includes Risk Mitigation

Population Subgroup (a)	Chronic PAD (Fg/kg/day)	Chronic Food Exposure with FHE (Fg/kg/day) (b)	Max. Water Exposure (Fg/kg/day) (c)	Surface Water Monitoring Data (Fg/L)	Ground Water SCI-GROW (excluding well contamination) (Fg/L)	Chronic DWLOC (Fg/L) (d,e,f)
U.S. Population	0.3	0.008	0.292	0.026	0.007 to 0.103	10
All Infants (< 1 Year)	0.03	0.01	0.02			0.2
Children (1-6 years)	0.03	0.015	0.015			0.15
Females (13-50 years)	0.03	0.006	0.024			0.72

(a) In addition to the U.S. population (all seasons), the most highly exposed subgroup within each of the infants, children, female groups is listed.
(b) Values are from Table 4 (and rounded).
(c) Maximum Water Exposure (Fg/kg/day) = Chronic PAD (Fg/kg/day) - [Chronic Food Exposure + Chronic Residential Exposure (Fg/kg/day) (if applicable)]. Chronic residential uses were not considered based on mitigation options.
(d) DWLOC (Fg/L) = Maximum water exposure (Fg/kg/day) x body wt (kg) ÷ water consumed daily(L/day)]
(e) HED default body weights are: general U.S. population, 70 kg; adult females, 60 kg; and infants/children, 10 kg.
(f) HED default daily drinking water rates are 2 L/day for adults and 1 L/day for children.

As noted previously, long-term exposure to chlorpyrifos as a result of well contamination from termiticide use could potentially result in exposures of concern. However, the groundwater risk estimates from well contamination resulting from termiticide use are highly localized. The implementation of PR 96-7 for termiticides has reduced the reported incidence of groundwater contamination resulting from termiticide treatments.

Although not all of the risk estimates for termiticide use achieve a margin of exposure of 1000, the Agency believes that individuals are unlikely to experience adverse health effects from the termiticide use of chlorpyrifos. This conclusion is based on: the public health protective assumptions; the 1000 fold safety factor; and the additional 3 to 10 fold cushion between the NOAEL and the LOAEL. Mitigation measures will further reduce exposures and risk associated with the termiticide use. For example, the removal of whole house barrier treatment addressed the exposures of most concern. It is expected that the limited spot and localized treatment, and pre-construction treatments would represent less exposure and risk. In conclusion, based on the mitigation plan, and best professional and scientific judgement, the Agency concludes that the chronic aggregate risk including termiticide use, does not raise a concern.

6.0 Cumulative Exposure and Risks

The Food Quality Protection Act (1996) stipulates that when determining the safety of a pesticide chemical, EPA shall base its assessment of the risk posed by the chemical on, among other things, available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances that have a common mechanism of toxicity. The reason for consideration of other substances is due to the possibility that low-level exposures to multiple chemical substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the other substances individually. A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Chlorpyrifos is a member of the organophosphate (OP) class of pesticides. All pesticides of this class contain phosphorus and other members of this class of pesticides are numerous and include azinphos methyl, chlorpyrifos-methyl, diazinon, dichlorvos, dicrotophos, dimethoate, disulfoton, methamidophos, methidathion, monocrotophos, oxydemeton methyl, phorate, phosmet, and pirimiphos-methyl to name a few. EPA considers organophosphates to express toxicity through a common biochemical interaction with cholinesterase which may lead to a myriad of cholinergic effects and, consequently the organophosphate pesticides should be considered as a group when performing cumulative risk assessments. HED recently published the final guidance that it now uses for identifying substances that have a common mechanism of toxicity (FR 64(24) 5796-5799, February 5, 1999).

HED has recently developed a framework that it proposes to use for conducting cumulative risk assessments on substances that have a common mechanism of toxicity. This framework was presented to the SAP. The SAP was in general agreement with the framework, and made recommendations for improving it. HED plans to release the proposed framework for public comment in March 2000. The framework is available from the Internet at: http://www.epa.gov/scipoly/. In the framework it is stated that a cumulative risk assessment of substances that cause a common toxic effect by a common mechanism will not be conducted until an aggregate exposure assessment of each substance has been completed. The framework is expected to be finalized by the fall of 2000. When the methods are completed and peer reviewed, EPA will proceed with a cumulative assessment of the organophosphates. The current assessment addressed only the risks posed by chlorpyrifos.

7.0 Confirmatory Data

Additional data requirements have been identified in the attached Science Chapters and are summarized here.

7.1 Toxicology Data for OPPTS Guidelines

HED has recommended and the registrant has developed a protocol for a Repeated Exposure Neurotoxicity Study of Sensory Electrophysiology. This study will also include measurement of neurotoxic esterase (NTE). It is expected that this would be a 28 day 2 dose, oral exposure study. In addition to the neurophysiological and neurochemical measures, neuropathological assessment focused on central/peripheral axonopathic changes associated with OPIDN (organophosphate-induced delayed neuropathy should also be performed). This is special study for which no single EPA guideline provides complete guidance. EPA has a guideline for 28 day hen studies of organophosphates that may cause OPIDN that includes guidance for neuropathology and NTE measurements (US EPA 1998; 870.6100). EPA has a guideline for examining peripheral nerve function (US EPA 85-SS1998; 870.6850) and a guideline for sensory evoked potentials (US EPA 1998; 870.6855). The current protocol for this special study has been developed by the registrant working voluntarily in conjunction with EPA. While EPA has not required this study, EPA maintains the right to require further study, based on concerns for potential health effects, consistent with its obligations under FIFRA.

7.2 Product and Residue Chemistry Data for OPPTS Guidelines

7.2.1 Product Chemistry

Forty (40) MP's have been identified. Guideline 830.6314 data requirements remain outstanding for the DAS 99% T. Data remain outstanding for all other chlorpyrifos MPs; for many MPs no product chemistry data have been submitted. The reregistration guidelines for

product chemistry data requirements are complete, provided that the registrants submit the data required in the attached summary tables for the chlorpyrifos MPs, and either certify that the suppliers of starting materials and the manufacturing processes for the chlorpyrifos technicals and manufacturing-use products have not changed since the last comprehensive product chemistry review or submit complete updated product chemistry data packages.

7.2.2 Residue Chemistry

The following confirmatory data requirements and/or label revisions for magnitude of the residue in plants (Guideline 860.1500) remain outstanding or are now required:

- For asparagus, no additional residue data are required. However, a label revision is needed. The maximum equivalent rate of 1.9 lb ai/A specified by a homeowner-use label (EPA Reg. No. 62719-56) should be adjusted to reflect the maximum registered rate of 1.0 lb ai/A for which adequate residue data are available. In a letter to the Agency dated 5/8/95 the registrant committed to correcting the label directions to 1.0 lb ai/A at the next label printing.

- For corn, label restrictions prohibiting feeding of silage, forage, or fodder to meat or dairy animals are not practical and must be removed from SLN DE930004 and FL940003 labels. Additional data must be submitted to determine if established tolerances on corn forage and fodder are adequate for these uses. Alternatively, these SLN uses may be canceled.

- For cotton, feeding restrictions for gin trash (gin by-products) are not practical and must be removed from product labels. Appropriate tolerances for cotton gin by-products must be proposed. The proposal must be supported by adequate residue data conducted according to the maximum use patterns.

- For crops grown solely for seed (clover, and grasses), tolerance proposals and adequate field residue data are required to support SLN (Section 24-c) uses. The Oregon Clover Association has indicated that it will support chlorpyrifos SLN (OR850032) use on clover grown for seed. The requirements specified in the Addendum to the Chlorpyrifos SRR remain outstanding. For grasses grown for seed, appropriate tolerances for residues of chlorpyrifos *per se* in/on grass forage and hay must be proposed. The proposal must be supported by adequate residue data conducted according to the maximum use patterns specified by NV940002, and OR94032.

Alternatively, these SLN uses may be canceled.

- For mint, Table 1 (OPPTS Test Guidelines 860, August 1996) requires data for peppermint and spearmint tops (leaves and stems). Mint hay is no longer considered a RAC. Additional data are required for peppermint and spearmint tops (leaves and stems).

- For peppers, the requirements specified by the Addendum to the Chlorpyrifos SRR to submit English translations of labels for all products that permit use of chlorpyrifos on peppers imported to the U.S. have not been fulfilled. Chlorpyrifos use on peppers was approved at the issuance of the SRR, SLN (FL920007, FL920009, GA930003, and GA930004).

- For sorghum, data are required for aspirated grain fractions.

- For the tree nuts group (almonds, filberts, pecans, and walnuts), the Addendum to the Chlorpyrifos SRR did not require additional data to support the established crop group tolerance. However, an examination of the recently amended labels for the 4 lb/gal EC formulation (EPA Reg. Nos. 62719-23 and 62719-220) indicated that a maximum seasonal rate of 10 lb ai/A was inadvertently approved for pecans. The available residue data, reflecting combined residues of chlorpyrifos and TCP in/on pecans and other representative members of this crop group, only support a maximum seasonal rate of 5 lb ai/A. If the registrant wishes to support a seasonal rate of 10 lb ai/A, then additional data are required. Alternatively, the labels for pecans may be revised to reflect a maximum seasonal rate of 5 lb ai/A. In a letter to the Agency dated 5/8/95, DAS stated that they would modify labels to reflect a maximal seasonal use rate of 5 lb ai/A for pecans at the next label printing. The latest approved label for Lorsban 4E (EPA Reg. No. 62719-220), dated 4/8/96 did not include this modification. The labels should be revised or appropriate residue data supplied.

- For wheat, data are required for aspirated grain fractions.

[Note: The field trial data submitted for asparagus, apples, sugar beets, and tree nuts depict combined residues of chlorpyrifos and TCP. In the absence of adequate data depicting chlorpyrifos *per se* on the commodities of these crops, the established tolerances, for tolerance reassessment purposes, should remain at the existing levels. It is the registrant's prerogative to petition the Agency and submit additional field residue data depicting chlorpyrifos *per se* in/on these crops if tolerance-level reductions or lower anticipated residue calculations are desired.]

GLN 860.1520: Magnitude of the Residue in Processed Food/Feed

According to Table 1 (August 1996) OPPTS 860.1000 Test Guidelines residue data for sorghum flour are not needed at this time because it is used exclusively as a component of drywall, and not as a food or animal feed item, in the US. However, because 50% of the worldwide

sorghum production is used for human consumption, data may be needed at a later time.

The requirements for processing data on alfalfa meal are waived because residue data indicate that levels of chlorpyrifos *per se* are not likely to exceed the established tolerance in alfalfa hay following tests conducted according to registered uses. In addition, no sweet corn processing data are required since adequate corn forage data are available.

The available processing data for apples and sugar beets depict combined residues of chlorpyrifos and TCP. In the absence of adequate data depicting chlorpyrifos *per se* on the processed commodities of these crops, the established feed additive tolerances, for tolerance reassessment purposes, should remain at the existing levels. It is the registrant's prerogative to petition the Agency and submit additional processing data depicting chlorpyrifos *per se* in/on these commodities if tolerance-level reductions or lower anticipated residue calculations are desired.

GLNs 860.1850 and 860.1900: Confined/Field Rotational Crops

Provided that DAS modifies all labels for its chlorpyrifos containing products to limit application to 5 lb ai/A/season on those crops where rotation to another crop could occur (as was stated in their letter to the Agency dated 8/12/94), HED will not require field rotational crop studies. Furthermore, a 30 day plant back interval for rotational crops would then be appropriate.

7.3 Occupational Exposure Data for OPPTS Guidelines

HED has insufficient data for the following agricultural handler scenarios:

- seed treatment uses
- dip applications (e.g., preplant peaches)
- dry bulk fertilizer applications to citrus orchard floors

These scenarios are of concern given the results from the other scenarios assessed.

For postapplication agricultural worker exposures, there is insufficient information (e.g., timing of applications -- dormant/bark versus foliar treatments) and exposure data to assess postapplication activities for ornamental and soil incorporated uses. The data needed to assess these uses include ornamental dislodgeable foliar residues in greenhouses and biological monitoring data for reentry into treated areas with soil directed applications.

In addition, HED could not evaluate the postapplication exposures and risks associated with use of insecticidal dust products due to an absence of chemical-specific data or recommended procedures in the Residential SOPs. Nevertheless, HED has concerns about the use of these products based on the low MOEs calculated using the surrogate data from the scientific literature for residents or workers that could apply these products. HED recommends that the registrant provide additional information on the potential post-application residential exposures associated with these products.

HED requests additional data for indoor crack, crevice and spot uses of chlorpyrifos. Specifically, HED requests treated room residue data for floors, furniture and other surfaces available for contact by children for both chlorpyrifos, and its primary degradation metabolite, 3,5,6-TCP following multiple treatments. Additionally, HED requests chlorpyrifos air measurements in treated rooms following multiple treatments (i.e., at a minimum 3 treatments 7 days apart). Residue data for 3,5,6-TCP are important due to the potential for accumulation and persistence of this environmental degradate.

HED requests confirmatory air monitoring data immediately following ground-based fogger application due to potential concern for short-term inhalation exposures.

In addition, HED requests exposure and/or environmental data for all registered products and/or uses that are not assessed in this risk assessment.

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APPENDIX A: Sensitivity/Susceptibility of the Young

The following summary has been extracted from the following report: "Chlorpyrifos Children's Hazard: Sensitivity and Susceptibility" HED Doc No. 014074, March 28, 2000. The entire document is also an appendix to the April 6, 2000 HIARC report (which is an attachment to the risk assessment).

The weight of evidence provides appreciable support for the increased sensitivity of the young compared to adult rats to the neurotoxic effects of chlorpyrifos and for the susceptibility of the developing brain to chlorpyrifos. A number of different rat studies clearly demonstrate that at a given oral dose the young rat will respond more to the anticholinesterase effects of chlorpyrifos (as defined biochemically and behaviorally) than adult animals. The differential found between pups and adult animals is a function of the treatment dose, duration of treatment, timing of treatment (*i.e.*, developmental stage) and of measurements (*i.e.*, time to peak effect), and the toxicological endpoint examined. At high acute doses, chlorpyrifos is fatal to the rat pup, but produces no lethality and little to no behavioral changes in the adult rat (*e.g.*, LD_{10} and MTD doses = neonate-15 mg/kg; adult-136 and 100 mg/kg, respectively). At the LD_{10} or MTD doses neonates are up to ~5-fold more sensitive than adult rats to ChEI (brain and blood) and clinical/behavioral effects. Furthermore, at a single treatment of 15 mg/kg, the down-regulation of the cholinergic (muscarinic) receptors was more extensive in the pups than in adults treated with 80 mg/kg. The magnitude of change, the effective time points, and the brain regions involved were different in pups versus adult rats. This suggests that the cholinergic receptors are more readily altered in the pup following chlorpyrifos treatment. Although the consequence of this is unknown, cholinergic receptors play an important role in normal brain development.

The increase in sensitivity between young and adult animals appears to occur at acute doses below 15 mg/kg. The study by Zheng *et al.* (2000) using lower dose levels (ranging from 0.15 mg/kg to 15 mg/day) provides cholinesterase inhibition (ChEI) data in 7-day old animals and adult male rats showing a greater sensitivity (up to ~3-fold for RBC and plasma, and perhaps at least 5-fold for brain) of pups compared with adult males. In the Zheng *et al.* study, the adult did not respond at the high dose of 15 mg/kg for brain ChEI. Thus, a difference in response greater than 5-fold can not be ruled out. Because of the lack of data, the extent of differences in brain ChEI between pups and the pregnant female rat remains uncertain. Although the young animal appears to recover at least two times faster than the adult animal from the ChEI induced by acute chlorpyrifos treatment, other toxicities (*e.g.,* delays in brain development, behavioral effects) may persist or appear at later times.

Repeated dosing with chlorpyrifos does not appear to result in an increase in brain or blood ChEI in neonates relative to adults with one exception. Based on $ED_{50's}$, there is a 1.5-fold difference in the response of PND 7 pups to brain ChEI compared to adult males (Zheng *et al.,* 2000). In contrast to the rapid recovery from ChEI observed with acute chlorpyrifos treatments of neonates (Pope and Liu, 1997), repeated dosing with

chlorpyrifos (every other day, 11 treatments during PND 1 to PND 21) indicates ChEI persists for ~9 to >19 days depending on the dose administered (Tang *et al.,* 1999). Body weight changes and behavioral effects occur at ~3-fold lower doses in neonates versus adult rats with repeated treatments of chlorpyrifos doses equal to or above 3 mg/kg/day.

It is apparent that cholinesterase activity is inhibited in the fetus if the dam is treated with a chlorpyrifos dose which can be absorbed by the fetus. The magnitude of brain, plasma, and RBC ChEI in the fetus is less or equal to that observed in dams with acute or repeated treatments of dams with chlorpyrifos. The lack of an apparent differential response of the fetus (or neonate with repeated dosing) versus the maternal system to treatment of dams with chlorpyrifos may be due to the increased new synthesis or more rapid turnover of inhibited molecules of cholinesterases in the fetal brain than in the adult (Lassiter *et al*., 1998; Mortensen *et al*., 1998).

Differences in detoxification between the young and adults may explain the increased sensitivity of exposed pups to chlorpyrifos toxicity. Chlorpyrifos and its oxon *(i.e.*, the anticholinesterase metabolite) are detoxified by binding to carboxlyesterases and hydrolysis by A-esterases. The young animal has minimal activity of these detoxification enzymes compared to adult animals. The precise influence of these enzymes on sensitivity to chlorpyrifos treatment has not been established. Because detoxification enzyme activities increase with age, the enzymatic profile of newborn rats raises concern that the newborn may be even more sensitive than older neonates to an acute chlorpyrifos treatment. There is some evidence (albeit at high doses) that suggests that the magnitude of the differential sensitivity between young and adult animals depends on the age of the animal. Based on the LD_{10} data in Zheng *et al.* and from the ChEI data in Zheng *et al.* and Moser and Padilla (1998), the order of sensitivity is PND 7 > PND 17 > PND 27 > adult female > adult male. Therefore, given that 7-day old rats are the youngest animals evaluated to date, it is uncertain whether the magnitude of differential sensitivity would be greater with pups exposed earlier than 7 days.

The developmental neurotoxicity study, which involved treatment of dams with 5, 1, or 0.3 mg/kg/day chlorpyrifos from GD 6 through lactation day 11 (Hoberman, 1998a,b), offspring were observed to have alterations in brain structure that are suggestive of a developmental defect that may predispose the neonate to unique adverse consequences. In this study, morphometric measurements in PND 11 pups of the high dose included, decreases in anterior to posterior measurements of the cerebellum, reduced height of the cerebellum, decreased thickness of the parietal cortex, and decreased thickness of the hippocampal gyrus. These effects at the high dose occurred in the presence of maternal toxicity (*e.g*., maximum brain, RBC and plasma ChEI) but in the absence of effects on body weights, food consumption, pregnancy parameters, or deaths among the dams. In mid- and high-dose PND 66 offspring, effects on brain structure included marginal but statistically significant decreases in the thickness of the parietal cortex and non-significant decreases in the thickness of the hippocampal gyrus. This difference in the qualitative severity of the findings seen in adult and neonatal animals is indicative of susceptibility of the offspring. It is also important to note that morphometric evaluation of the low-dose

brains was not conducted. So it is not known whether alterations are occurring at lower doses.

Additionally, a number of the treatment-related findings in the offspring appear to be delayed in expression of perturbations in earlier neurological development, because functional and morphological changes are observed at study termination (~PND 61 - 66), approximately 50 - 55 days after cessation of maternal dosing. At the high dose, these findings included increased motor activity in females at PND 61, alterations in auditory startle measurements (increased latency to peak response and decreased peak response amplitudes) at PND 62, and morphometric alterations in the parietal cortex and hippocampal gyrus on PND 66.

A variety of *in vitro* and *in vivo* studies published in the peer reviewed literature show that chlorpyrifos can alter macromolecular synthesis, neuronal activity, neurotransmitter levels, neurite outgrowth and branching, and cell signaling in the developing rat brain (reviewed by Slotkin, 1999). Although these studies did not include accompanying measures of direct adverse effects (*e.g.,* functional effects) but rather used biomarkers, they nevertheless raise concern that chlorpyrifos potentially can affect processes occurring in both early and late developmental periods of brain growth that influence cell replication and differentiation needed for normal function. Although the data primarily come from one laboratory, multiple studies from this group have shown a consistency in the different responses measured. Furthermore, several of the key responses observed are highly significant and robust (*e.g.,* effects on norepinephrine turnover, DNA synthesis, adenylyl cyclase transduction). Also, the responses reported tend to have little variability in the data. Finally, effects on the developing brain reported in the literature are consistent with the morphometric changes observed in the guideline developmental neurotoxicity study by Hoberman (1998) even though a direct linkage of effects can not be made. The available data suggest a selective action of chlorpyrifos on the developing brain, given the regional and temporal pattern of responses. Thus, it seems unlikely that the observed effects are due to nonspecific toxicity.

Although there are strengths of these studies, there are also some limitations and questions raised which are not addressed by the results. As discussed above, the mechanism of action for chlorpyrifos in the developing brain is unclear. Also, the *in vivo* studies using macromolecular biomarkers have primarily been conducted using the subcutaneous injection (SC) route of exposure and DMSO as the vehicle. It should be noted that DMSO controls were conducted in all the studies. DMSO would result in a rapid uptake and full absorption of the compound. Compounds administered via SC injection enter directly into the general circulation and bypass hepatic metabolism once, thus bypassing hepatic activation of chlorpyrifos to its active metabolite chlorpyrifos-oxon. The SC route of exposure can not be reliably compared to the oral route given the lack of pharmacokinetic data on this dosing regime. Also, this is not a pathway of human exposure. Thus the DMSO-SC dosing regime makes quantitative interpretation and extrapolation of the results problematic. Nevertheless, these studies still provide important qualitative information on the potential for chlorpyrifos to affect neurodevelopmental processes. Cholinesterase inhibition was not measured in most of these studies except for Song *et al.* (1997). In that study, no extreme cholinesterase inhibition is found in the brainstem at the low dose used in the study: approximately 20-25% cholinesterase

inhibition is found when 1 mg/kg of chlorpyrifos is administered during PND 1-4 and cholinesterase activity (measured 24 hours after the last dose) is almost completely recovered by 10 days of age (Song *et al.*, 1997). Given that key effects in the postnatal brain are found at the low dose, the concern of a rapid delivery of a toxic dose with this standard dosing regime is reduced. Also, no significant changes in body or brain weight and no mortality occurs with this dosing regime (1 mg/kg at PND 1-4 or 5 mg/kg at PND 11-14). Additionally, it should be noted that chlorpyrifos is rapidly absorbed and transported to the brain with oral dosing (Mendrala and Brzak, 1998). Thus, the findings derived from the SC/DMSO dosing regime can not be discounted as an artifact of the vehicle and route of exposure and raise concerns for the unique susceptibility of the young.

The mechanism(s) of action for the chlorpyrifos-induced changes (*e.g.,* macromolecular synthesis, cell signaling) is/are unclear. However, given that these effects can be found after intracisternal injection of chlorpyrifos, with *in vitro* TCP treatment, and *in vitro* PC12 cell cultures with limited capability to activate chlorpyrifos to its ChE-inhibiting oxon, raises the issue of whether these effects can occur independent of cholinesterase inhibition. Although it is not possible to link each effect reported with another effect or with a functional outcome, the data show a consistent pattern of the potential for chlorpyrifos to produce qualitatively different effects in the central nervous system (CNS) of young versus adult animals. Potential implications of the effects include alteration of synaptic responses that are programmed by neural input, disruption of cell replication and differentiation, and temporary or persistent delays in the development of CNS structures.

In conclusion, the weight of the evidence raises concern for an increase in both the sensitivity and susceptibility of the fetus or young animal to adverse biochemical, morphological, or behavioral alterations from chlorpyrifos treatment during brain development. With respect to cholinesterase inhibition, an increase in sensitivity of the young compared to adults was seen all along the dose response curve, even at relatively low doses. There is a clear differential response (2- to ~5-fold) in the young compared to the adult animal after an acute treatment to a relatively low dose of chlorpyrifos. There is also increased sensitivity found after repeated dosing (up to 9-fold), but at the LD_{10} and MTD. It is important to point out that an uncertainty remains concerning the magnitude of the differential response, given that newborn animals (less than PND 7) have not been characterized for sensitivity. Results of multiple studies have consistently shown that the developing brain is susceptible to chlorpyrifos treatment. Effects on the developing CNS that are indicative of the unique susceptibility to the young animal include changes in macromolecular synthesis, altered cell signaling and muscarinic receptor down-regulation, as well as morphological alterations in brain development. An uncertainty remains regarding the NOAELs for the susceptibility effects. The effects observed raise a high degree of concern that the fetus or young animal is particularly susceptible to adverse outcome if exposed to chlorpyrifos.

AN ABSTRACT OF THE THESIS OF

G. Lalith Mahendra Aponso for the degree of Doctor of Philosophy in Toxicology presented on July 30, 2001. Title: Exposure and Health Risk Assessment for Farmers Occupationally Exposed to Chlorpyrifos in Sri Lanka; and Drinking Water and House Dust Analysis for Chlorpyrifos.

Abstract approved: _____
Ian J. Tinsley

This study was designed to assess chlorpyrifos exposure of a group of farmers by determining internal dose associated with a given application of this insecticide. This involved the monitoring of urinary levels of 3,5,6 trichloro-2-pyridinol (TCP), the major metabolite of chlorpyrifos. Incidental exposure was evaluated by determining the levels of chlorpyrifos and TCP in drinking water and house dust.

Nineteen full-time farmers from Kandy district, Sri Lanka, growing long-squash or bitter melon during the 2000 vegetable season (April-June) participated in the study. Information concerning their health history, agricultural practices, family background and pesticide-related issues were obtained using a questionnaire. All farmers used knapsack sprayers for applying a chlorpyrifos EC formulation. The amount of chemical applied, time required, and the safety precautions used were noted.

One urine sample was taken prior to application followed by three samples a day for 5 days post application from each farmer. Urine samples were extracted with hexane and analyzed for TCP using a gas chromatograph fitted with an electron capture detector. The limit of detection for TCP in urine was 6ng/mL.

TCP levels peaked within 24 hours post application and returned to the baseline after 5 days. Total TCP voided ranged from 71 to 299μg (average of 190.4ug) per 5g of creatinine, equivalent to a calculated internal dose of 0.0021-0.0084mg/kg (average 0.0055mg/kg) chlorpyrifos. It was assumed that 90% of the internal dose was voided in urine in 5 days. The dermal dose ranged from 4.8 to 19.6μg/cm^2 on exposed skin. The elimination half-life of the urinary TCP metabolite was 31.2 hours. The internal dose was correlated with the amount of active ingredient used ($p< 5 \times 10^{-7}$), the use of leaky tanks ($p<0.005$), and the use of protective clothing ($p<0.005$). Hazard quotient for cholinesterase inhibition based on the EPA reference dose for chlorpyrifos ranged from 0.8 to 2.7 and the margin of safety from 3.6 to 14.3 for the exposed farmers. None of the farmers were found to have symptoms of acute or sub-chronic poisoning in the medical examination carried out at the end of the season.

Drinking water was collected from three wells, and dust was collected as floor wipes from three houses located adjacent to treated areas. Chlorpyrifos was not detected in well water at levels that could be quantitated (minimum detection limit was 7ng/L). TCP was detected in well water 9 to 10ng/mL. Although some chromatograms suggest the presence of chlorpyrifos in some house dust samples (minimum detection limit 13ppb), a comparison of the responses on two different columns did not provide convincing evidence for the presence of chlorpyrifos. Failure to detect significant amount of chlorpyrifos in water and house dust was probably due to rapid break down due to high soil temperature and pH. Water and house dust did not add to the farmers' occupational exposure.

Exposure and Health Risk Assessment for Farmers Occupationally Exposed to Chlorpyrifos in Sri Lanka; and Drinking Water and House Dust Analysis for Chlorpyrifos

by

G. Lalith M. Aponso

A THESIS

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in partial fulfillment of

the requirement for the

degree of

Doctor of Philosophy

Presented July 30, 2001

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Doctor of Philosophy thesis of G. Lalith M. Aponso presented on July 30, 2001

APPROVED

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Major Professor, representing Environmental and Molecular Toxicology

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Dean of Graduate School

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G. Lalith Mahendra Aponso, Author

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The various co-authors of the chapters are listed together with major contributions to the study.

Ian J. Tinsley	Technical support; critical review of the experimental design, data and manuscript; guidance; patience.
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Bobby Lopper	Technical support; method improvement; and guidance.
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DEDICATION

This thesis is dedicated to my loving son, Savinda Aponso, and daughter, Hiruni Marsha Aponso, with encouragement for their future studies.

EXPOSURE AND HEALTH RISK ASSESSMENT FOR FARMERS OCCUPATIONALLY EXPOSED TO CHLORPYRIFOS IN SRI LANKA; AND DRINKING WATER AND HOUSE DUST ANALYSIS FOR CHLORPYRIFOS

CHAPTER 1

INTRODUCTION

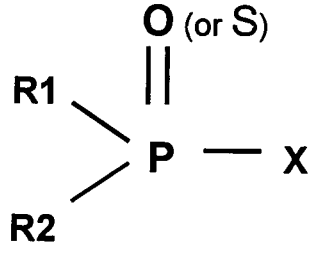
Pesticide use on agricultural crops is considered an efficient method for safeguarding against yield losses due to pests in a given ecosystem. Application of these substances poses a health risk to non-target species such as humans, domestic animals, and wildlife. Pesticide applicators, neighboring communities, and consumers of the produce can be at risk by oral, dermal, or inhalation exposure. The level of the risk depends on the inherent toxicity of the agent of interest and the magnitude of exposure.

PESTICIDES

A pesticide is defined as any substance or mixture of substances intended for destroying, repelling, or mitigating the activity of any pest. It is also described as any physical, chemical, or biological agent that will kill an undesirable plant or animal pest. Pesticides are mainly classified into different classes according to their usage such as insecticides, herbicides, fungicides, rodenticides, etc. Pesticides belong to different chemical classes such as organophosporus (OP), chlorinated hydrocarbons, bipiridyl, aminoacids, etc. Most of the OP pesticides are insecticides. Chemicals also can be assigned to one of five toxicity classes based on acute toxicity as indicated by the LD_{50} (oral, dermal) or LC_{50} (inhalation) values.

ORGANOPHOSPHORUS COMPOUNDS

OP compounds are widely used as pesticides throughout the world. These compounds are also used as plasticizers, lubricants, petroleum additives, and chemical warfare agents. OPs command the largest segment (more than 1/3) of the total $6.1 billion insecticide market worldwide. Over 89 million acres of the United States are sprayed annually with OP insecticides. Effectiveness as pesticides and rapid biodegradability favors the use of OP compounds. These compounds are normally esters, amides, or thiol derivatives of phosphoric or phosphonic acid. The general structure of an OP compound is given in Figure 2.1.



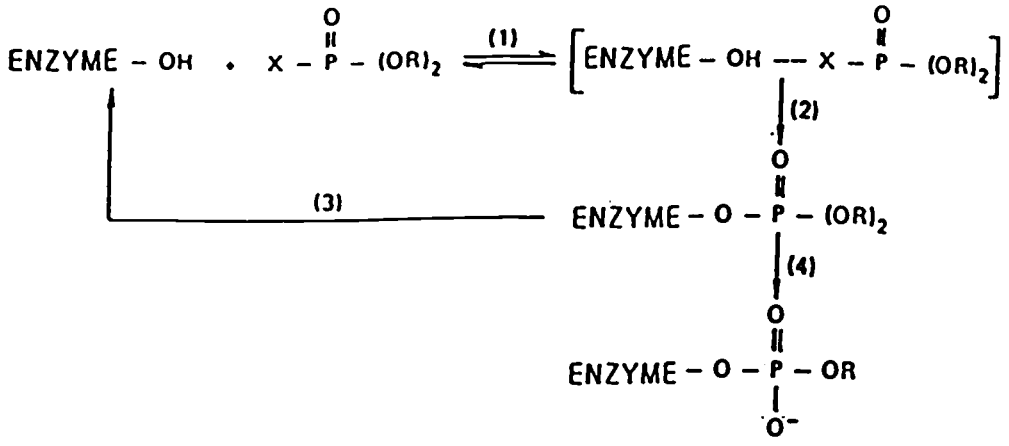
R1 and R2 are usually methyl or ethyl group, both of which may be bound directly to phosphorus (in phosphinates) or linked via -O- or -S- (in phosphates). R1 may be bound directly and R2 bonded via one of the above groups (phosphonates). In phosphoramidates, carbon is linked to phosphorus through a -NH group. X is called the leaving group, and it is usually bound via an -O- or -S- molecule. (WHO, 1986)

Figure 1.1: General structure of organophosphorus compound

TOXICITY OF ORGANOPHOSPHORUS COMPOUNDS

The toxicity of OP compounds is primarily due to the inhibition of acetylcholinesterase (AChE), an enzyme necessary for the normal function of the central and peripheral nervous system. AChE is a serine protease that hydrolyses the neuro-transmitter, acetylcholine (ACh). AChE (True cholinesterase) and pseudocholinesterase belong to an enzyme class called choline ester hydrolases (Ballantyne and Marrs, 1992). AChE is found postsynaptically in central and peripheral cholinergic synapses, including the preganglionic autonomic synapses and postaganglionic parasympathetic synapses (Palmer, 1980). It is also found at the motor end plate in the neuromuscular junction and is also associated with erythrocytes (Ballantyne and Marrs, 1992). Esterase enzymes such as AChE are inhibited by phosphorylation upon acute exposure to an OP compounds (Figure 1.2). Inhibition of AChE activity in nerve tissue leads to a range of effects resulting in dysfunction of central and peripheral nervous systems by over stimulating the target tissue and culminating in respiratory failure and death. Misra et al. (1985) reported that occupational exposure of applicators to the OP pesticide fenthion resulted in headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%). A study on acute, chronic, and accidental exposure of OP pesticides to agricultural workers in California indicated that significant number of workers had signs of exposure (Brown et al., 1989).

AChE present on erythrocytes and cholinesterase (ChE) found in plasma does not have any known function in blood. Inhibition of erythrocyte AChE is proportional to the level of exposure and the affinity of the compound for the enzyme. In contrast, plasma ChE is more sensitive to inhibitors. Plasma ChE is inhibited to a greater degree by OP compounds such as chlorpyrifos, diazinon, dichlorvos, and malathion while the erythrocyte enzyme is more sensitive to dimefox, parathion, and parathion-methyl (Hays, 1982). Inhibition of blood ChE is not commonly considered as an adverse effect.



(1) Formation of Michaelis complex

(2) Phosphorylation of the enzyme

(3) Reactivation reaction

(4) Aging

Figure 1.2: Inhibition of an esterase enzyme by OP compounds

Blood AChE inhibition and the level of metabolites found in urine have been used as biomarkers for exposure (Knaak et al., 1979; Franklin et al., 1981) and biomarkers for adverse effects (Padilla et al., 1996) to OP or carbamate (another group of anti-cholinesterase pesticides) insecticides. Depression of blood AChE correlates with effects in the target tissue (generally, central or peripheral nervous system depending on the affinity), but the exact relationship depends on the time after exposure, the tissue, and the insecticide. The best correlation is achieved during maximal cholinesterase inhibition either after an acute dose or during repeated dosing. During maximal inhibition, the response in whole blood, plasma, and erythrocytes will exhibit good correlation with the target tissue, but this relation is not observed during initial exposure and recovery phases. In the recovery phase, erythrocyte and whole blood cholinesterase activity may lag behind recovery in the target tissue (Padilla et al., 1996). Further, they reported that some OP compounds show a linear relationship between blood cholinesterase inhibition and presence of clinical signs or change in behavior. As an example, chlorpyrifos-treated rats showed a linear relationship between blood ChE inhibition and motor activity impairment, but the ChE has to be depressed to at least 15% of control for a significant response. Animals fed with aldicarb (a carbamate pesticide) and paraxon (an OP pesticide) needed 50-60% inhibition of ChE to initiate a response. In a study using rats and beagle dogs, McCollister et al. (1974) reported plasma and erythrocyte ChE are depressed by smaller doses of chlorpyrifos than inhibit in brain ChE or produce signs of toxicity. Thus, changes in plasma and erythrocyte ChE have been used most frequently as a screen in evaluating an individual's exposure to chlorpyrifos (Nolan et al., 84). Gibson et al. (1998) suggested that plasma cholinesterase activity is the most sensitive indicator of exposure to chlorpyrifos.

Some OPs induce a delayed neuropathy, which develops weeks after a single exposure. Manifestation of OP induced delayed neuropathy differs among species with locomotor effects prominent in humans and hens for

example, but lacking in laboratory rats. Potential for the development of this progressive and irreversible neuropathy is determined by the capability of the OP to significantly and irreversibly inhibit neuropathic target esterases (NTE). Relative inhibition of NTE and AChE shortly after exposure may be used to distinguish the likelihood of causing delayed neuropathy or acute toxicity following exposure to OP compounds (Ehrich, 1996). A stable covalent bond at active sites of the enzymes causes the irreversible inhibition, and the process called aging further enhances stability of the bond when one of the alkyl groups of the diethylester is lost. Senanayake and Karalliedde (1987) described the acute neurotoxic effects during the cholinergic phase of OP insecticide poisoning and delayed neurotoxic effects that appeared 2-3 weeks later. In this study, they described patients appearing to have a distinct clinical entity (a so-called intermediate syndrome) that developed after the acute chlolinergic crisis and before the expected onset of the delayed neuropathy. OP pesticides reported to cause delayed neuropathy in man are mipafox (Bidstrup et al., 1953), leptophos (Xintaras et al., 1978), methamidophos (Senanayake and Johnson, 1982), trichlorphon (Shiraishi et al., 1977), trichlornat (Jedrzejowska et al., 1980), EPN (Xintaras and Burg, (1980) and chlorpyrifos (Lotti and Morretto, 1986).

The American Conference of Governmental Industrial Hygienist (ACGIH, 1995-1996) has established threshold limit values (TLV) to protect workers from exposure to solvents. TLV is the airborne concentration of a substance a worker could be exposed to daily without exhibiting adverse effects. There are three types of TLVs: (1) the time weighted average (TWA), which is a value for an 8-hr working day and for a 40-hr work week; (2) the short term exposure limits (STEL) is a value for a short period of time (usually 15 min); (3) the ceiling (TLV-C) is a value that should not be exceeded even briefly. The dermal exposure TLV and STEL for chlorpyrifos are 0.2 and 20 mg per cubic meter, respectively (USDHHS, 1997).

SRI LANKA

Sri Lanka is located in the Indian Ocean, 29 km off the southeastern coast of India. Its total area is 65,610 square kilometers and it is positioned between 5^0 and 10^0 north latitude. Sri Lanka has a warm climate moderated by ocean winds and considerable moisture. The mean temperature ranges from 15.8 °C in the central highlands to a high of 29 °C in the northeast coast, but some areas may reach 37 °C during July and August. Humidity is typically higher in the southwest and mountainous areas, and it varies with the seasonal patterns of rainfall. The country is divided climatically into a wet zone (southwestern quarter), a dry zone (north and eastern areas), and an intermediate zone (between wet and dry zone), based on annual precipitation. Average rainfalls are 250 cm, 120 cm, and 190 cm, respectively.

Over sixty percent of the 19 million population depends on agriculture or agricultural based industries. A majority of the vegetable farms are found in villages and most farmers own a land area of less than one acre. Crops grown depend on the rainfall and availability of irrigation water. In areas with no irrigation, rice is cultivated in the main rainy season and vegetables are grown during minor rains. Recently, a focus on high yield crops such as rice and other vegetables have resulted in an increased demand for fertilizers and pesticides. Currently, pesticides are used as the major method of pest control. Pesticide importation, formulation, distribution, storage and other related activities are monitored by the Control of Pesticide Act No. 33 of 1980 and it's amendment of 1994. Monitoring and controlling the use of pesticides in the field are lagging behind due to a lack of personnel in the pesticide control authority and in the agricultural extension service.

In Sri Lanka, insecticides are mainly used for pest control in agriculture and malaria vector control. According to the Registrar of Pesticides (the pesticide regulatory authority) of Sri Lanka, total technical grade insecticides (active ingredient) and formulated products imported to the country during

1998 were 393 and 3131 metric tons, respectively. Sixty one percent of total insecticides were OPs and 19% were carbamates (another anti-cholinesterase pesticides). Major contributions to OP's were from chlorpyrifos 40% emulsifiable concentrate (EC), dimethoate 40% EC, and diazinon 5% granules (G). Quantities of these formulated products used during 1999 were 198, 175 and 278 metric tons, respectively.

It has been estimated that 95% of fatal pesticide poisonings occur in developing countries, many of which are in the Asia-Pacific region. Agriculture-based economies, availability of pesticides, socioeconomic problems, lack of adequate protective clothing and limited treatment facilities are some of the factors contributing to the high intoxication and mortality (Fernando, 1995). In Sri Lanka, the number of hospital admissions due to OP pesticide poisoning in 1992 was 11,439, which was 73% of total pesticide poisonings (Fernando, 1995). Death records indicated that OPs are the major pesticides causing poisoning (Fernando, 1995; Senanayake and Peiris, 1995). In another study, Jayaratnam (1987) indicated that 5 out of 1000 agricultural workers in Sri Lanka were hospitalized yearly due to pesticide poisoning from occupational exposure. Many cases of intoxication due to occupational exposure may not require admission to a hospital and therefore go unreported.

Most of the farmers do not have adequate knowledge of the hazards of pesticides. Inappropriate activities such as using hands for mixing pesticides in knapsack sprayers, accidental spilling, leaking tanks, smelling pesticides, lack of protective clothing and many other factors may lead to increased dermal and inhalation exposure. Farmers of poor economic condition do not have the resources to replace or maintain spray equipment for optimal function. Knapsack sprayers are the primary equipment used for pesticide application. These sprayers are designed to be held with the nozzle in front of the operator and hence the area in front is sprayed, which causes the applicator to continually walk through the sprayed crop.

Due to the high cost of agricultural inputs and climatic uncertainties, farming is not a highly profitable business in Sri Lanka. Therefore, family labor and exchanged labor (with neighboring farm-families) is used for cultivation to minimize cost of production. Spray drift, could carry pesticide residue to drinking water sources and to nearby houses. In addition, pesticide contaminated clothes, dirty spray equipment, and improper storage conditions in houses may pose an exposure risk to children and other members of the house who are not involved in agricultural activities.

Rice cultivation is fed either by rain or channel irrigation depending on the monsoon season. Heavy monsoon rains cause runoff carrying soil and pesticide residues downstream which ends up in lakes and rivers. Exceeding recommended application rates especially on lowland crops such as rice, may accumulate the environmental impact. A study performed among vegetable farmers in three growing regions indicated that 63.5% of the farmers use more than the recommended dose of pesticides, 85.7% applied pesticides before the appearance of pests, and 8% sprayed pesticides prior to marketing (Chandrasekera et al., 1985).

In some agricultural areas purified tap water is not available and well water is used for drinking. Use of plastic pesticide containers for storing water and the use of river or lake-water for bathing and laundry may lead to a significant exposure to pesticides and other environmental contaminants.

CHLORPYRIFOS

Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioat) (CAS Register No. 2921-88-2) is a broad-spectrum OP insecticide widely used in agriculture and residential pest control. The structure of chlorpyrifos is given in Figure 1.3. According to the United States Environmental Protection Agency (US EPA), chlorpyrifos is registered for use on pests in fruits, nuts and

vegetables. Department of Agriculture Sri Lanka recommends chlorpyrifos 40EC formulation for pest control in rice and vegetables and as a treatment for soil termites.

As with the other OP compounds, the principle action of chlorpyrifos and its bio-activated product, chlorpyrifos-oxon, is inhibition of neural AChE (Namba et al., 1971). An oral LD_{50} of 152 mg/kg was reported for female mice and 169 mg/kg for female rats fed chlorpyrifos (Berteau and Deen, 1978). Oral LD_{50} values for male and female rats ranged from 118 to 245 mg/kg (Gaines, 1969). In a study of the pharmacokinetics of chlorpyrifos in human, no cholinergic signs were manifested at oral doses of 0.5 mg/kg, even though plasma cholinesterase activity was depressed to 15%. At this dose no toxicity signs were observed in any volunteers. Subchronic NOEL for human plasma cholinesterase activity depression was 0.03 mg/kg/day (Coulston et al., 1972).

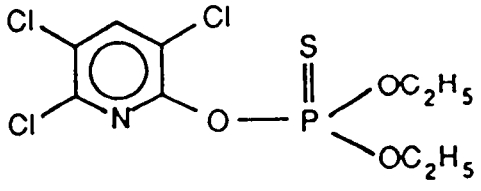


Figure 1.3: Structure of chlorpyrifos

DISTRIBUTION AND METABOLISM OF CHLORPYRIFOS

Distribution of orally administered ^{14}C-labeled chlorpyrifos has been investigated in male Wister rats (Smith et al., 1967) and Hereford crossbred heifers (Dishburger et al., 1977). Results of both studies indicated chlorpyrifos was distributed to all organs but liberation from fat was slower (half-life [$t_{1/2}$] is 67hr) than other tissues ($t_{1/2}$ is 10-16hr). Distribution of dermally exposed chlorpyrifos was investigated in goats (Cheng et al., 1989), mice (Shah et al., 1981) and bovine (Claborn et al., 1968; Ivery et al., 1972). The parent compound was reported to distribute throughout the body, but concentrations were comparatively higher in blood, liver and fat.

Microsomal cytochrome P-450 enzymes catalyze the oxidative desulfuration, bioactivation of chlorpyrifos to form chlorpyrifos-oxon (oxon) in rat and mouse liver (Sultatos and Murphy, 1983a; Ma and Chambers, 1994). In vitro studies showed that the oxon is 400 times more active than chlorpyrifos as an inhibitor of cholinesterase (Sultatos et al.,1982). Both chlorpyrifos and it's oxon are rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol (TCP) probably by A-esterase in humans (Sultatos and Murphy, (1983a, 1983b), rats and goats (Glas, 1981). Studies using liver perfusion have shown that both bioactivation and detoxification occurs rapidly hence only TCP can be detected in the hepatic effluent once steady-state conditions are reached (Sultatos and Murphy, 1983a, 1983b). Hydrolysis of oxon by A-esterase is probably the more common rout of detoxification, since TCP or a conjugate of TCP is the major metabolite of chlorpyrifos in humans (Nolen et al., 1984) and rodents (Bakke et al., 1976; Smith, 1967). The principle route of excretion in humans is through urine. This rapid conjugation and elimination reaction reduces occurrence of adverse health effects. Fate of chlorpyrifos in the human body is illustrated in Figure 2.4.

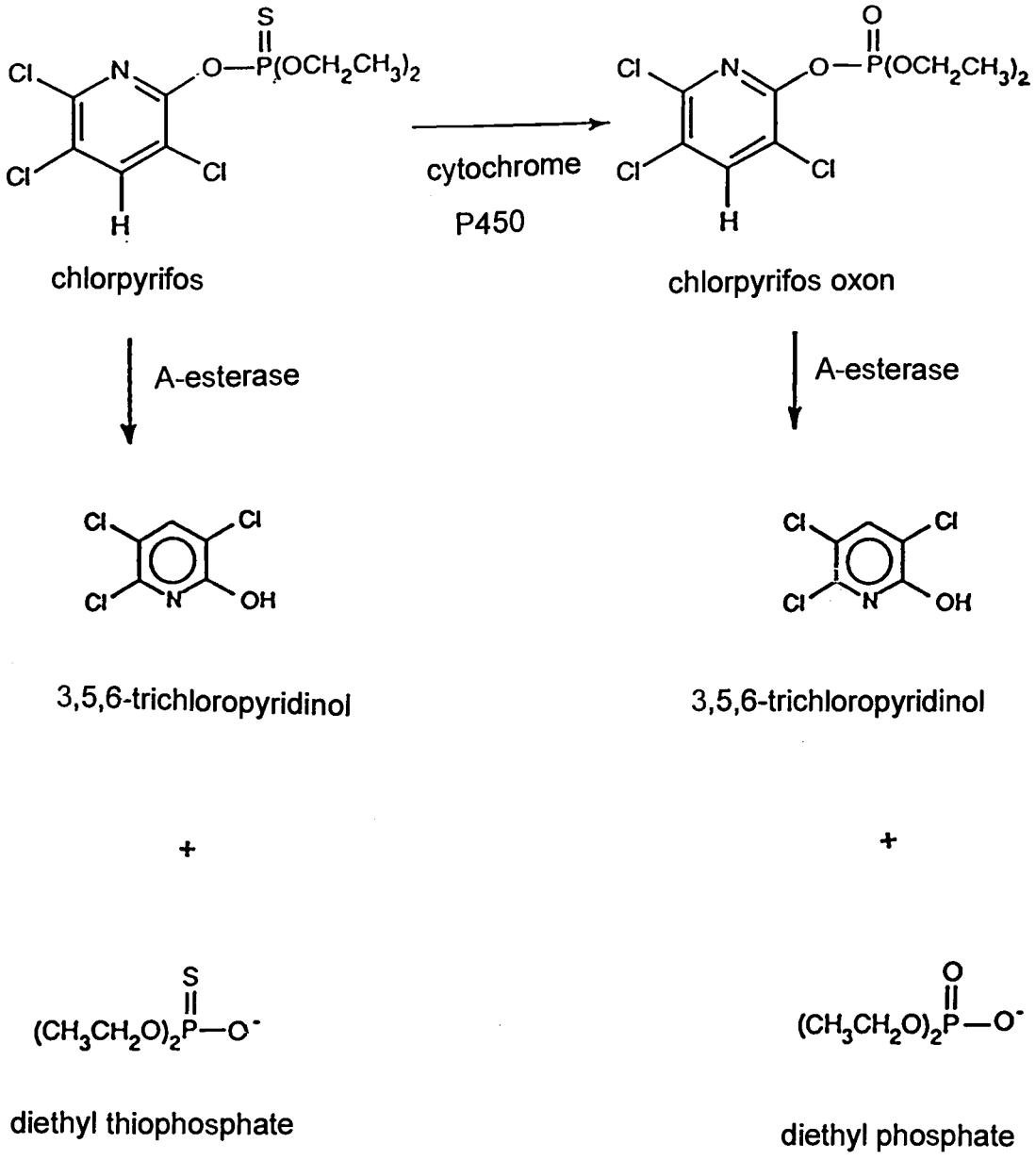


Figure 1.4: Fate of chlorpyrifos in human

In 1984, Nolan et al. studied the pharmacokinects of chlorpyrifos in human volunteers. They reported that chlorpyrifos is rapidly metabolized to TCP and excreted into urine in humans. Maximum blood concentration of this major metabolite was observed 6hr after oral exposure and 24hr after dermal exposure of chlorpyrifos. The mean half-life for the elimination of TCP from the blood was 26.9hr following both oral and dermal doses. The amount recovered as metabolites from urine was equivalent to 70% of the oral dose and 1.28% of the dermal dose (within 5 days).

Griffin et al. (1999) studied the oral and dermal absorption of chlorpyrifos using five human volunteers age range 26-45 years. All the subjects were given an oral dose of 1 mg of analytical grade chlorpyrifos. This dose is half that which would be absorbed if a subject were exposed to the Health and Safety Executive occupational exposure standard of 0.2 mg/m^3 over an 8hr period. Blood samples were taken over a 24hr period, and the total voided volume of urine was collected over 100hr. They reported that TCP, diethylphosphate, and diethylthiophosphate are the specific urinary metabolites of chlorpyrifos. In this study, the total diethylphosphate and diethylthiophosphate voided were determined for each volunteer as a biomarker of exposure. Ninety three percent of the oral dose was recovered in urine. Four weeks later, 28 mg of chlorpyrifos was administered dermally to the same volunteers over an 8hr period. Unabsorbed compound was washed off after this period. One percent of the dermal dose was recovered as metabolites in urine. This dermal dose was unable to depress plasma ChE, but detectable levels of metabolites were found in urine. Therefore, the authors concluded that urinary metabolites are the more sensitive biomarker of exposure. The US EPA reference dose (RfD) for oral exposure to chlorpyrifos is 0.003 mg/kg/day (US EPA, 1997). This RfD was obtained from a NOEL of 0.03 mg/kg (oral) for ChE inhibition (Coulston et al., 1972) and an uncertainty factor of 10 for human variability.

ENVIRONMENTAL FATE OF CHLORPYRIFOS

Chlorpyrifos as granules is applied in significant quantities directly to soil or sprayed as liquid on plants, often at times when irrigation is employed to supplement natural rainfall. Both rainfall and irrigation can contribute significantly to chemical transport in runoff. Chlorpyrifos will degrade by both biotic and abiotic transformation processes in terrestrial and aquatic environments. In soil, water, plants and animals, the major pathway of abiotic and biotic degradation involves cleavage of the phosphorothioate ester bond (Racke, 1993) to form TCP (Figure 1.5). In the environment, TCP is degraded via photolysis with an aqueous half-life of about 4 min in surface water at 40^0N latitude (Dilling et al., 1984) and microbial degradation with an average half-life of 73 days at 25 ^{0}C (Bidlack, 1976). In terrestrial ecosystems, chlorpyrifos rapidly dissipates from plant foliage, with an observed half-life of 1 to 9 days (Racke, 1993). Chlorpyrifos dissipated at a moderate rate when incorporate into the soil profile with a half-lives of 33-56 days in Califonia, Michigan, and Illinois (Fontaine et al., 1987). However, dissipation from soil surfaces occurs rapidly compared to deep soil. Half-lives of 9-11 days have been noted for fallow soil surfaces and from 7-9 days from turf grass surfaces following spray application at sites in Indiana and Florida (Racke and Robb, 1993).

In aquatic ecosystems, chlorpyrifos is removed from the water column via hydrolysis, biodegradation, sorption to sediments, volatilization and photodegradation. Hydrolysis half-lives in sterile distilled water have been reported to range 16-72 days at pH 5-9, while laboratory photolysis half-lives of 30-52 days have been reported (Racke, 1993). Degradation half-lives in sediment water under aerobic and anaerobic conditions have been reported as 22-51 and 39-200 days, respectively in laboratory conditions (Racke, 1993).

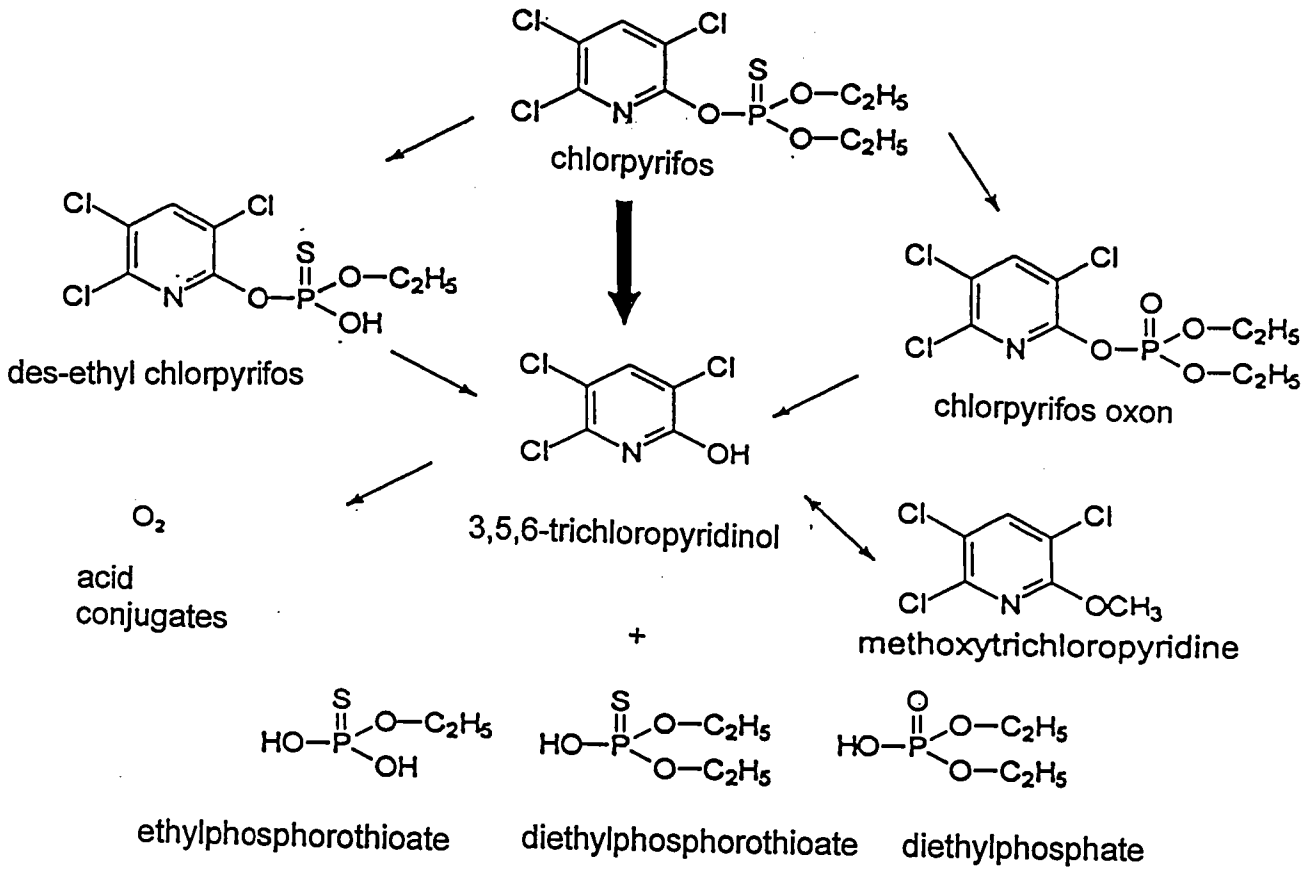


Figure 1.5: Environmental transformations of chlorpyrifos (adapted from Racke 1993)

RISK ASSESSMENT

Risk: Risk is a function of dose and the inherent toxicity of the compound. In general, risk is defined as the possibility of injury, harm, or other adverse and unwanted effects. Risks are commonplace in all of our lives. Risk assessments are conducted to estimate how much damage or injury can be expected from exposure to a given risk agent and to assist in judging whether these consequences are severe enough to warrant more intensive management or regulation. In the health, safety, and environmental fields, risk is usually identified as the likelihood that individuals (or population) will incur increased incidences of adverse effects such as disabling injury, disease, or death. Risk is frequently expressed in probability terms such as some number of additional deaths over a lifetime in a population of exposed people. Historically, a risk of less than 10^{-6} in magnitude has been considered acceptable in cancer incidence. The methods and sequence of steps involved in conducting a risk assessment vary with the kind of risk, i.e., threshold or non-threshold. In general, this process consists of four steps such as hazard identification, exposure assessment, dose-response assessment, and an integrative risk characterization.

a) Hazard Identification: This initial step of risk assessment seeks to identify the adverse health effect that can be caused by exposure to the chemical being studied. An adverse health effect can be temporary, permanent, or life threatening.

b) Exposure assessment: The objective of the exposure assessment is to estimate the route and magnitude of exposures to the chemicals of concern. Since risk is proportional to magnitude of exposure, this estimation is essential (and the more difficult parameter to assess) to calculate risk factors for individuals or to a population.

c) Dose response assessment: In this step, the extent of adverse effects resulting from a given level of exposure to a risk agent are evaluated, usually on experimental animals. This dose response relationship provides a toxicological reference that is used to estimate the likelihood or severity of adverse effect for the exposed individuals.

d) Risk characterization: This is the final step of risk assessment, which involves assembling prior analysis components to determine risk. In this step, the toxicity and exposure assessment are summarized and integrated into quantitative and qualitative expressions of risk. To characterize potential non-carcinogenic effects, comparisons are made between dose and toxicity values to provide a margin of safety. Risk quotient (or hazard quotient) is a function of dose (exposure level) and the inherent toxicity of the chemical.

OBJECTIVES

This study is focused on assessing the exposure and consequential risk for pesticide applicators by determining internal dose. Farmers in Sri Lanka take minimal safety precautions in handling pesticides. This may lead to high exposure levels via dermal, oral, and inhalation routes while handling concentrate, mixing, or applying pesticides in the field. Hence, farmers are at a high risk of pesticide exposure and poisoning. High temperature and humid conditions, which discourage the use of protective clothing, poor personal hygiene, and lack of knowledge of pesticide hazards of pesticides increases the potential for exposure. The main objective is to use urinary TCP levels (pre and post application) to calculate the internal dose. Since total urine collection is not practical under occupational conditions, TCP levels in urine are expressed as per gram of creatinine clearance.

Most residents in the farming community drink well water because purified tap water is unavailable. These wells and residence houses of farmers are located close to the farming land, where water and house floors could be contaminated by pesticide spray drift. The second objective of this study is to analyze drinking water and house dust for the parent compound and the major metabolite to assess potential secondary exposure.

Another objective of this study is to conduct a survey to understand personal details, cultivation practices, health status, and other relevant information about the participants which might influence exposure before the experiment. Finally, the adverse health effects caused by chlorpyrifos application by a medical examination after the experiment.

CHAPTER 2

EXPOSURE AND RISK ASSESSMENT FOR FARMERS OCCUPATIONALLY EXPOSED TO INSECTICIDE CHLORPYRIFOS IN SRI LANKA

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ABSTRACT

Urinary levels of 3,5,6 trichloro-2-pyridinol (TCP, CAS 6515-38-4), the major metabolite of chlorpyrifos, were measured in farmers occupationally exposed to the parent compound, chlorpyrifos. This study was designed to assess the internal dose experienced and the risk for farmers who applied chlorpyrifos on their crops during the major vegetable season (April-June) of the year 2000. Nineteen full time farmers from an agricultural community in Kandy district, Sri Lanka, participated in the study. A questionnaire was used to record health history, personal information, agricultural information, family background, pesticide-related issues, and health status. One urine sample was taken before application and sampling continued for 5 days (three samples per day) after application. TCP levels in urine peaked in the first day post application, returning to the baseline by the end of the fifth day. Cumulative TCP voided ranged from 71 to 299μg (average of 190.4μg) per 5g of creatinine and was equivalent to an internal dose of 0.0021-0.0084 mg/kg (average 0.0055 mg/kg) chlorpyrifos assuming 90% of the internal dose was voided in urine in five days. TCP levels were correlated with the amount of active ingredient used ($p< 5 \times 10^{-7}$) and the use of leaky tanks (p=0.005) and protective clothing (p=0.005). Calculated dermal dose ranged from 4.8 to 19.6 $\mu g/cm^2$ on exposed skin. The elimination half-life of the urinary TCP metabolite was 31.2hr. The calculated hazard quotient for cholinesterase inhibition using the EPA reference dose for chlorpyrifos ranged from 0.8 to 2.7, and margin of safety ranged from 3.6 to 14.3 for the farmers. Parent compound was not detected in any of the urine samples. None of the farmers were found to have acute or sub-chronic symptoms in the medical examination carried out at the end of the season.

Farmers do get higher doses than the reference dose by occupational exposure. Slow dermal uptake, rapid metabolism, and elimination of the parent compound seem to protect against an acute response. The short

application times and long intervals between application, may also be protective.

INTRODUCTION

Chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyrdyl]) phosphorothioate (CAS Registry No. 2921-88-2) is a widely-used broad-spectrum insecticide recommended for use in many countries on various food crops and for the control of household insects. Chlorpyrifos is one of the most commonly used OP insecticides in agriculture with a high potential for inducing adverse health effects. Inhibition of AChE upon exposure and urinary 3,5,6-trichloro-2-pyrdynol (TCP) have been used as biological markers to assess chlorpyrifos exposure. Other agents such as carbamate compounds can inhibit AChE, but chlorpyrifos is one of only two insecticides that has TCP as a metabolite.

In a pharmacokinetics study, Nolan et al. (1980) reported on signs and symptoms of toxicity, changes in plasma and erythrocyte cholinesterase, and urinary TCP levels in six human volunteers administrated an oral dose (0.5 mg/kg) or dermal dose (5 mg/kg) of chlorpyrifos. In this study, the highest blood TCP concentrations of 0.93 μg/mL were reached 6h after an oral dose and 0.063 μg/mL 24h after a dermal dose. The average half-life ($t_{1/2}$) for TCP appearance in blood was 0.5h for oral and 22.5hr for dermal dose. Average TCP excreted in urine was 70±11% of the oral dose and 1.28±0.8% of the dermal. The mean $t_{1/2}$ of elimination of TCP from the blood was 26.9hr following both oral and dermal dose. Plasma cholinesterase was depressed 85% by the oral dose and 13% after the dermal dose. Erythrocyte ChE activity was essentially unchanged following the oral or dermal doses. Blood chlorpyrifos concentrations were less than 30 ng/mL, and no unchanged chlorpyrifos was found in the urine following either route of administration.

Griffin et al. (1999) determined the kinetics of elimination of urinary dialkylphosphate metabolites after oral and dermal exposure to human volunteers to doses of chlorpyrifos. Five volunteers ingested 1 mg (2852nmol) of chlorpyrifos, and 4 weeks later 28.59 mg (81567nmol) of chlorpyrifos was administrated to the skin of the same group by spreading 100μl of a

commercial preparation of chlorpyrifos (Durban 4, Dow Elanco), diluted in water on to an area of 78 cm^2 for 8hr. Total urine was collected over 100hr. It was observed that 93% of the oral dose and 1% of the dermal dose was recovered as urinary dialkylphosphate metabolites. Excretion after a dermal dose was delayed compared with the oral dose. The apparent elimination half-life of urinary dialkylphosphates after an oral dose was 15.5hr, and 30hr following the dermal dose. Plasma or erythrocyte cholinesterase activity was not depressed significantly by any of these doses. Urinary dialkylphosphate metabolites, which can be detected readily, are thus a more sensitive indicator of exposure.

Very limited studies are available for inhalation toxicology for chlorpyrifos. According to the United Stated Department of Health and Human Services (USDHHS) publication (1997) on toxicology profile for chlorpyrifos, no information is available on acute or sub-chronic inhalation exposure of chlorpyrifos to humans. In a chronic study, the prevalence of selected illnesses and symptoms in 175 employees involved in the production of chlorpyrifos were compared with 335 control matched subjects, but there was no significant difference found among groups (USDHHS, 1997). It was also reported that oral and inhalation exposure contributes to a greater internal dose than dermal absorption of chlorpyrifos. In mice, the inhalation LD50 of 94 mg/kg was determined after whole-body inhalation exposure to 6700-7900 mg/m^3 chlorpyrifos aerosol in xylene by varying the length of exposure from 27-50 min. Acute LD50 for virgin female Spague-Dawley rats similarly exposed to 5900-7500 mg/m^3 chlorpyrifos for varying length of time from 60 to 180 min was 78 mg/kg (Berteau and Deen, 1978).

OBJECTIVES

Chlorpyrifos is used widely to control agricultural pests in Sri Lanka. Farmers take minimal safety precautions in mixing and applying a pesticide. Protective clothing is rarely used because of the hot humid climate and, in most cases would not be available because of the farmers socioeconomic status. Farmers receive limited training and safety practices. The potential for exposure is obvious. This study designed to assess the exposure of farmers to chlorpyrifos by monitoring the urinary TCP levels after an application. Background information was completed to evaluate variables affecting exposure. The risk of a chlorpyrifos application to the farmer was determined using the internal dose.

MATERIAL AND METHOD

Field experiments: One of the main vegetable growing areas in Kandy district, Sri Lanka was selected for the study in 2000. Vegetables are grown during the minor rainy season (April to June) since no irrigation system is available. One of the cultivation sites selected is shown in Figure 2.1.

Farmers: Nineteen male farmers, 35-48 years of age participated in this study. Farmers were recommended by the Agricultural instructors and the head farmer (an on-site person employed by the Department of Agriculture) of the Kandy Provincial Department of Agriculture Sri Lanka. Selections were based primarily on the crops grown and the pesticides used to control insects. Farmers growing long squash (Figure 2.2) or bitter melon were chosen because the crop vines grow on canopies. Farmers completed questionnaire to provide background information on general health status, health history,

Figure 2.1: One of the areas selected for the study

method of pesticide application, area under crop of interest, cultural practices, level of education, and family background. Farmers were found to be in good health. None of the farmers were taking any medication for chronic health problems. They all agreed not to use chlorpyrifos for at least 10 days before the study and to collect urine samples as specified. The volume of chlorpyrifos concentrate used per tank mix, number of tanks sprayed, duration of application, protective measures used, method of cleaning spray tanks, personal hygiene and local weather conditions were recorded for each application. Average daytime temperature and relative humidity was 30-34 ^{0}C and 70-80%, respectively, during the study period.

Chemicals and Pesticides: Chlorpyrifos 40% EC; manufacturer & formulator Cheminova Agro A/S, Denmark, was a kind donation of BASF Finlay (Pvt.) Ltd., 186, Vauxhall Street, Colombo. TCP and chlorpyrifos standards were donated by Dow Agroscience, 9330 Zionsville Road, Indianapolis.

Pesticide application and sample collection: All farmers used their own or rented knapsack sprayers to apply pesticides. They applied pesticides on their crop using normal application techniques. Particular attention was given to collect the urine samples at the right time and bring them back to the laboratory. The head farmer of the area who had overall control of all the farmers was given the most responsible role in sample collection. This person provided the communication-bridge between the Department of Agriculture and the farmer. Two additional Agricultural Instructors from the Office of Registrar of Pesticides were also assigned to monitor applications and sample collection. Amber glass bottles (100 mL) were used to collect urine. All bottles were washed with hexane and methylene chloride before use. A volume of 100 mL of urine was collected from each farmer as the baseline (control) urine sample before application of chlorpyrifos. Since all the farmers apply pesticides in the morning, the first two samples were taken around 3pm and

Figure 2.2: Long squash plants and fruits

9pm on the same day of application, and the third sample required for the first day post application was collected from the first urination on the following day. The same cycle was repeated to collect 24hr samples for 5 days. Sample were returned to the laboratory daily and stored in the refrigerator until extracted.

Urine analysis for TCP: Conjugates of TCP were hydrolyzed by heating 10 mL of urine with 2 mL of concentrated H_2SO_4 acid for 1hr at 90 ^{0}C. TCP was extracted using 2 x 10 mL aliquots of hexane (Fisher Scientific, New Jersey, USA) and the two hexane extracts combined. Final volume was adjusted to 1 mL by evaporation under nitrogen gas (BOC group Inc. NJ). All samples and standards were derivatized with 5μL N,O-bis(trimethylsilyl) acetamide (BSA) prior to injection. Recovery was evaluated using four control urine samples spiked with different concentrations of TCP. Spiked concentrations and percent recoveries are listed in Table 2.2.

Table 2.1: Recovery of TCP from spiked urine

Amount spike (μg)	**Amount recovery (μg)**	**Percent recovery**
0.5	0.51	103
0.75	0.53	70
1.0	0.79	79
1.25	1.11	89

Average recovery 85.4%

GC analysis: A Varian 3600 gas chromatographic system with an electron capture detector was used for the analysis of TCP in urinary extracts. Two columns were fixed to the same injector port: a polar column, DB-XLB 30 m, 0.25 mm internal diameter, and 0.25μm film thickness and a non-polar column, DB-1 30 m, 0.25 mm internal diameter, and 0.25μm film thickness, manufactured by J&W scientific, USA. Data from both columns was used for confirmation. A 2μL split-less injection was used with a Varian auto sampler 8200. Temperatures at injector port and detector were 50^0 and 350 ^{0}C, respectively. The three-step column temperature program was 100^0, 190^0, and 300^0 for 6, 2.5, and 2.5 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 300 at 25 ^{0}C/min, respectively.

Sets of 30 samples along with two sets of six standards were used for each run. One set of standards was injected before the samples were injected and the other at the end. Two standards from the first set were injected between every three-sample injections. Standard curves generated for TCP were polynomial, and this shape was reproduced over all GC runs (Figure 2.3). Polynomial standard curve was close to linear and the slope was higher over the concentration range of 0.25-1.25 μg/mL. Hence the samples with TCP levels higher than 1.25 μg/mL were diluted with hexane to work in the linear range.

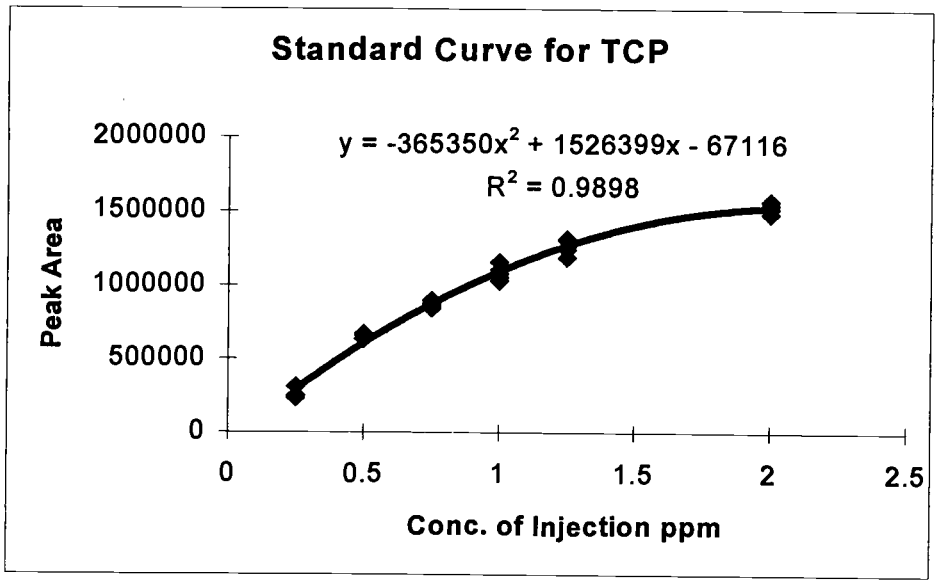


Figure 2.3: Standard curve generated for TCP

Instrument detection limits for urine analysis for TCP

Instrument noise = 0.5 mm (Figure 2.4)

Signal to Noise ratio = 3

Peak height of the 250 ng/mL standard = 113 mm

Calculation

Minimum measurable peak height = 0.5 mm x 3

= 1.5 mm

Minimum measurable concentration based on 1.5 mm peak

=(250 ng/mL/113 mm) x 1.5 mm

= 3.3 ng/mL

Instrument detection limit = 4 ng/mL

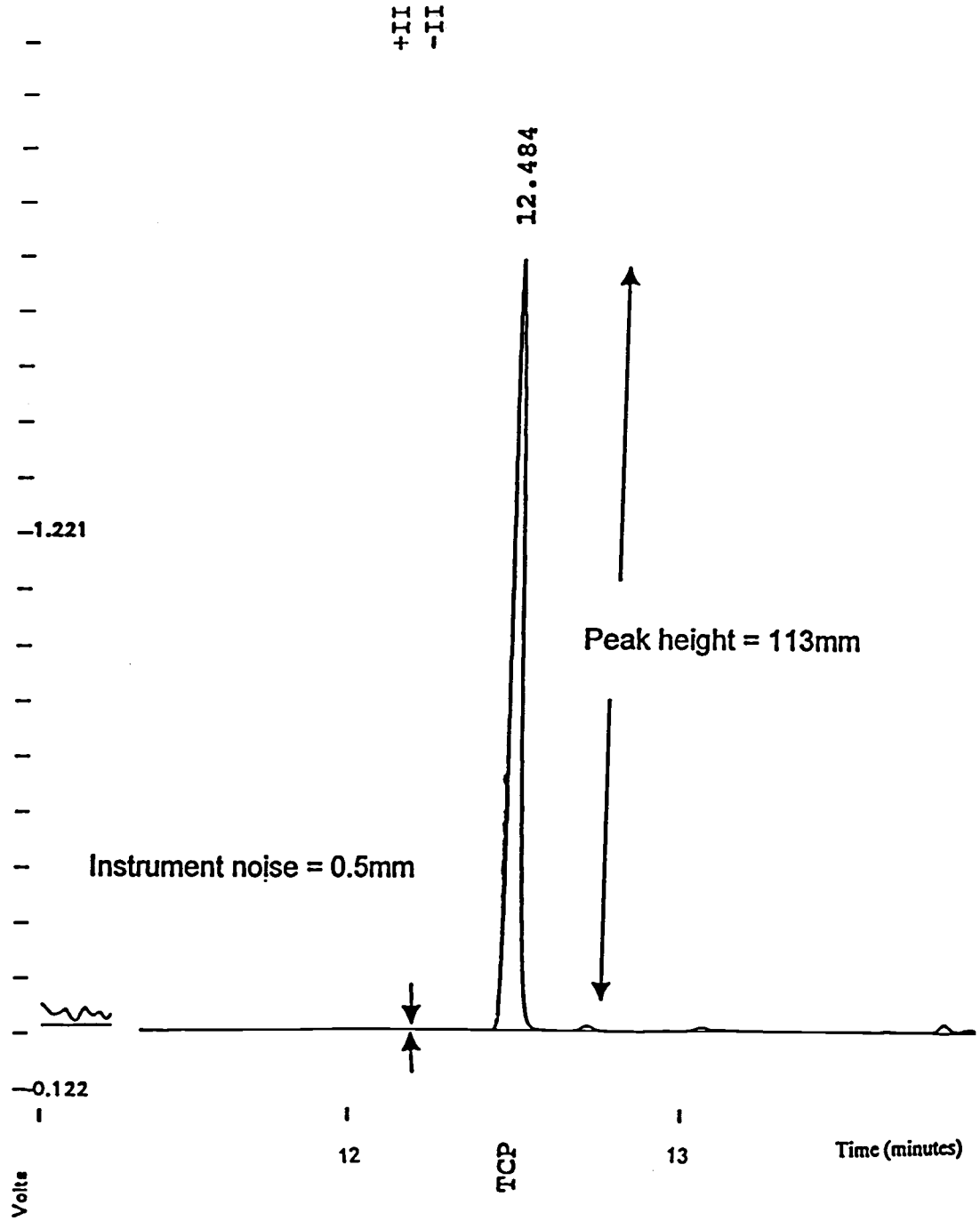


Figure 2.4: Chromatogram of 0.25 µg/mL TCP standard used to determine instrument detection limit in urine analysis for TCP (attenuation 1000)

Method detection limits for urine analysis for TCP

Method noise	= 8 mm (Figure 2.5)
Signal to noise ratio	= 3
Initial volume for the method	= 10 mL
Final volume for the method	= 1 mL
Peak height of the 250 ng/mL standard	= 113 mm

Calculation

Minimum measurable peak height = 8 mm x 3

= 24 mm

Minimum measurable concentration based on 24 mm peak

= (250 ng/mL /113 mm) x 24 mm

= 53 ng/mL

Since final volume is 1 mL , final concentration = 53 ng/mL

Initial volume is 10 mL; therefore,

Minimum amount of TCP in 10 mL of urine = 53 ng

Minimum concentration in urine = 53/10 ng/mL

Method detection limit = 6 ng/mL in urine

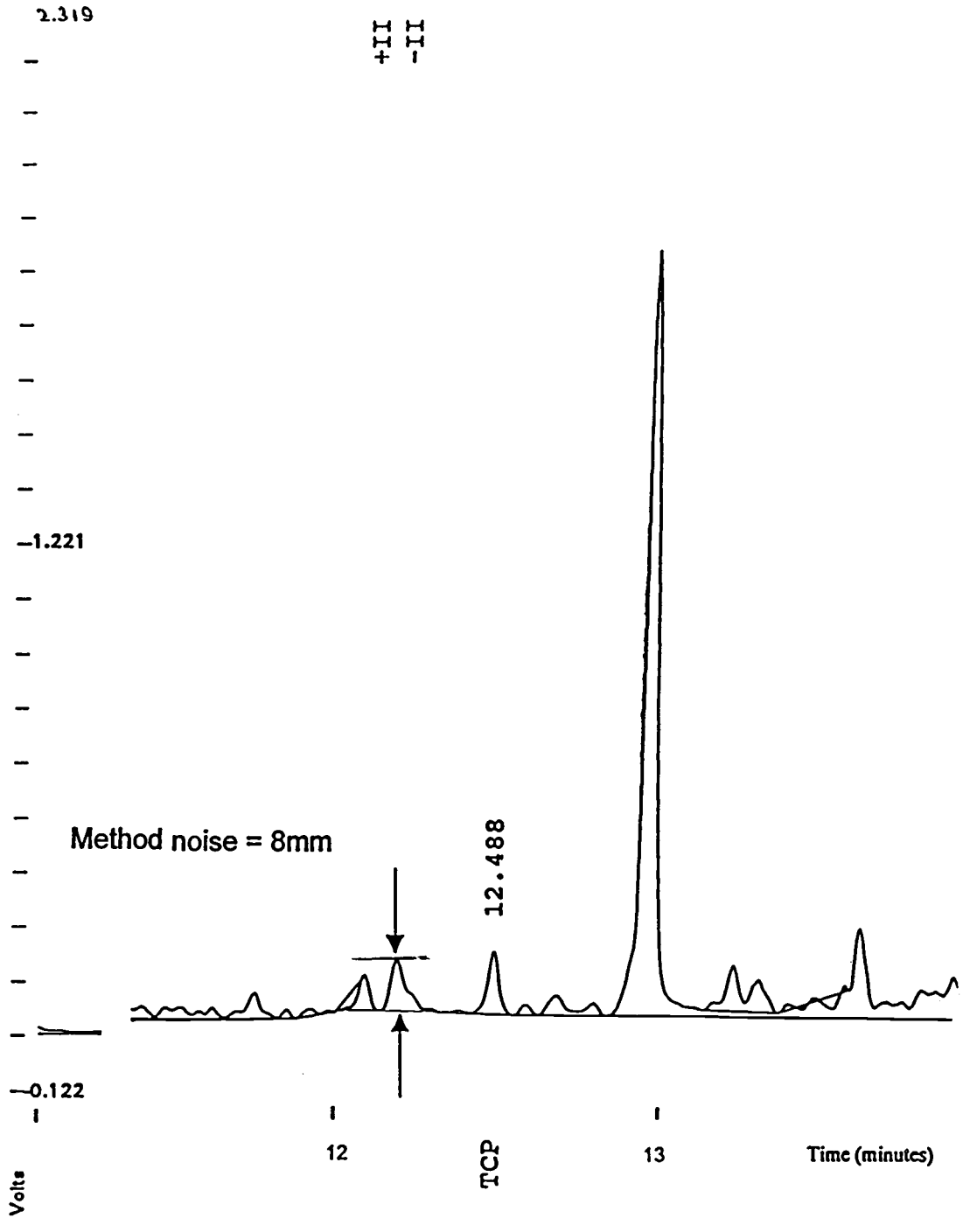


Figure 2.5: Chromatogram of blank urine extract used to determine method detection limit in urine analysis for TCP (attenuation 1000)

CREATININE ANALYSIS

Creatinine is a metabolic by-product of muscle, and an individual's muscle mass or lean body weight primarily determines its rate of production. It varies with age and gender, and for any given individual, the daily rate of creatinine production is assumed to be constant. Once creatinine is released from the muscle into plasma, it is eliminated almost exclusively by renal glomerular filtration. In the steady state, rate of creatinine production is equal to rate of elimination. In pharmacokinetic studies, urinary constituents are expressed as per gram of creatinine in urine.

Creatinine assay: Creatinine in alkaline solution reacts with picrate to form a colored complex, which increases the absorbance of the mixture. The change of absorbance over a specific time was measured in this assay (Henry, 1974).

Reagents:

1. Creatinine standard 2 mg/100ml (177μmol/L)
2. Picric acid 35 mmol/L
3. Sodium hydroxide 0.32 mmol/L

Procedure: Equal volumes of picric acid (35 mmol/L) and sodium hydroxide (0.32 mmol/L) were mixed as recommended (reagent mixture), and all reagents were stored in a refrigerator when not in use. Urine samples were diluted x50 with distilled water prior to the assay. Change in the absorbance at 492nm in first 2 min were recorded for the standard and samples. The instrument was set on kinetic mode for the assay. Standard solution or the samples were mixed with the reagent mixture in cuvettes as in Table 2.2 just before reading.

Table 2.2: Creatinine assay mixture

	Assay for standard	**Assay for samples**
Reagent mixture	2.0 mL	2.0 mL
Creatinine standard	0.2 mL	-
Sample	-	0.2 mL

Calculations:

A2-A1. Change in absorbance in standards($A_{standard}$)and samples ($A_{samples}$)

where A1= absorbance at 0 min

A2= absorbance at 2 min

$$\text{Urinary creatinine } \mu\text{mol/L} = \frac{A_{samples}}{A_{standard}} \times 177\ \mu\text{mol/L}$$

EXPOSED DERMAL AREA CALCULATION

Body surface area is a function of body weight (Wt) and height (Ht) of an individual. Mosteller's equation was used to calculate the total body surface area of the farmers (Mosteller, 1987). Areas of the different parts of the body of the farmers were calculated using the percentage values reported by Graber, 1997 (Figure 2.6, Table 2.3). Assumptions (a key) used to calculate uncovered skin area are given in Table 2.4.

Mosteller formula:

Body Surface Area (m^2)= (Height (cm) x Weight (kg)/3600) ^{½}

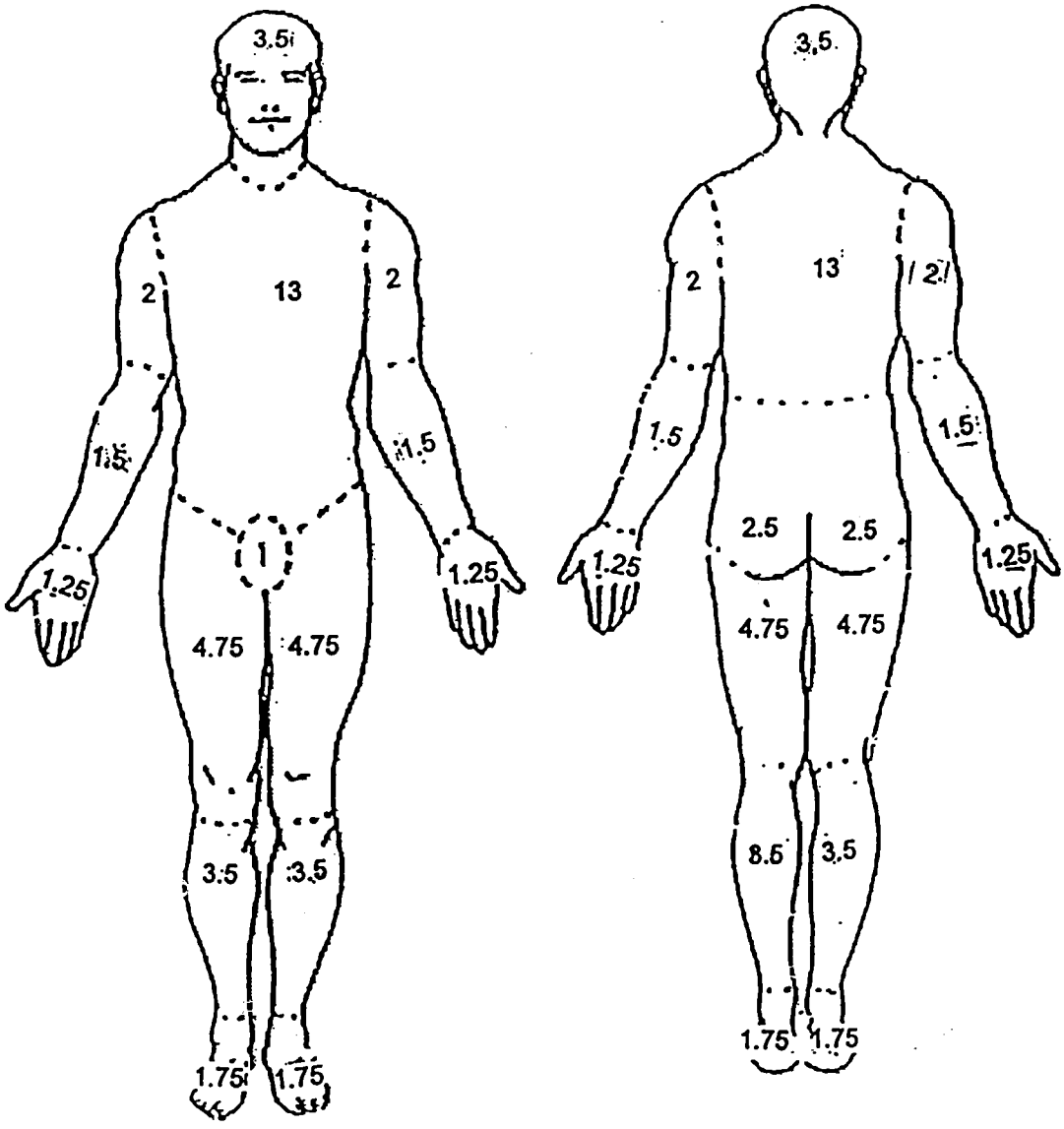


Figure 2.6: Area of different parts of the body

Table 2.3: Percent area of different parts of the body

Part of the Body	Percent of total area
Head	7
Neck	2
Anterior trunk	13
Posterior trunk	13
Right buttocks	2.5
Left buttocks	2.5
Genitalia	1
Right upper arm	4
Left upper arm	4
Right lower arm	3
Left lower arm	3
Right hand	2.5
Left hand	2.5
Right thigh	9.5
Left thigh	9.5
Right leg	7
Left leg	8
Right foot	3.5
Left foot	3.5

Table 2.4: A key used to calculate percent covered by clothing during application of chlorpyrifos

Protective clothing used	Percent covered
1	55
2	61
3	71
4	77
Hat	2.5
Gloves	5
Face cover	1.8

1= Short-sleeved shirt and covered up-to the knee

2= Long-sleeved shirt and covered up-to the knee

3= Short-sleeved shirt and long pans

4= Long-sleeved shirt and long pans

RESULTS

Questionnaire: Volume of pesticides applied on the crop varies for each farmer due to the fact that there were differences in area cultivated, density of the canopy (stage of the crop), mixing ratio and walking speed. Use of protective clothing and personal hygiene also varied. This variation among farmers led to different levels of individual exposure. The Department of Agriculture recommends using 28 mL of chlorpyrifos 40%EC formulated product per 16 liters of water, but about 30% of farmers used more than the recommended amount to achieve better pest control (Table 2.5). Many of the spray tanks were old and about 30% were leaking. Sprayers that are leaking result in an additional exposure through wet clothing. Half of the workers did not use a head cover. Personal information such as age, level of education body weight, size of family, alcohol consumption, and smoking habits are listed in Table 2.6. Only one farmer covered his face with a handkerchief. Many farmers bury left over pesticide in the bottle until it is used again. After pesticide application, there was about a 1hr delay before farmers washed in a stream.

Table 2.5: Agricultural details of the farmers

Farmer ID	Area cultivated (ac)	Chlorpyrifos 40% EC used (mL)	Number of tank loads	Spray mix (CPF* ml per 16L water)	Duration of application (hr)
F1	0.25	112	4	28	2.5
F2	0.25	168	4	42	3
F3	0.25	84	3	28	2.5
F4	0.3	112	4	42	3.5
F5	0.25	210	5	42	4
F6	0.3	210	5	42	4
F7	0.2	84	3	28	2.5
F8	0.3	140	5	28	4
F9	0.25	112	4	42	3
F10	0.25	210	5	42	4
F11	0.3	168	6	28	4.5
F12	0.25	112	4	28	3
F13	0.3	168	6	28	4
F14	0.25	112	4	28	3.5
F15	0.25	140	5	28	4.5
F16	0.2	84	3	28	2
F17	0.25	112	4	28	2.5
F18	0.25	140	5	28	4
F19	0.25	112	4	28	3

*CPF=chlorpyrifos

Table 2.6: Summery of personal details of the farmers

Farmer ID	Age	Level of education (grade)	Body weight (kg)	Members in the family	Alcohol consumption	Smoking
F1	35	8	65	3	Y	N
F2	38	10	70	4	Y	N
F3	41	8	75	3	Y	Y
F4	38	10	70	3	Y	Y
F5	47	8	73	3	Y	Y
F6	40	8	64	3	Y	Y
F7	41	8	65	4	Y	N
F8	35	10	68	3	N	N
F9	40	12	77	4	Y	Y
F10	42	6	81	3	Y	N
F11	41	6	73	3	Y	Y
F12	45	8	65	5	Y	N
F13	42	8	69	3	Y	Y
F14	39	6	73	4	Y	Y
F15	44	6	69	4	Y	Y
F16	39	10	70	2	Y	Y
F17	39	10	62	3	Y	Y
F18	42	8	63	3	N	Y
F19	46	8	65	4	Y	N

Pesticide application in the field: Chlorpyrifos was applied to the crop after each harvest. Farmers prepare pesticide spray mix by a stream which provides a convenient source of water. Most of them do not use gloves when handling concentrated pesticides. Figure 2.7 illustrates clothing used, preparation of spray mix for application, handing of concentrated pesticides, condition of some of the spray tanks, and the spray operation of some farmers. These conditions were similar for most of the farmers.

Figure 2.7: The process of applying pesticide on canopy

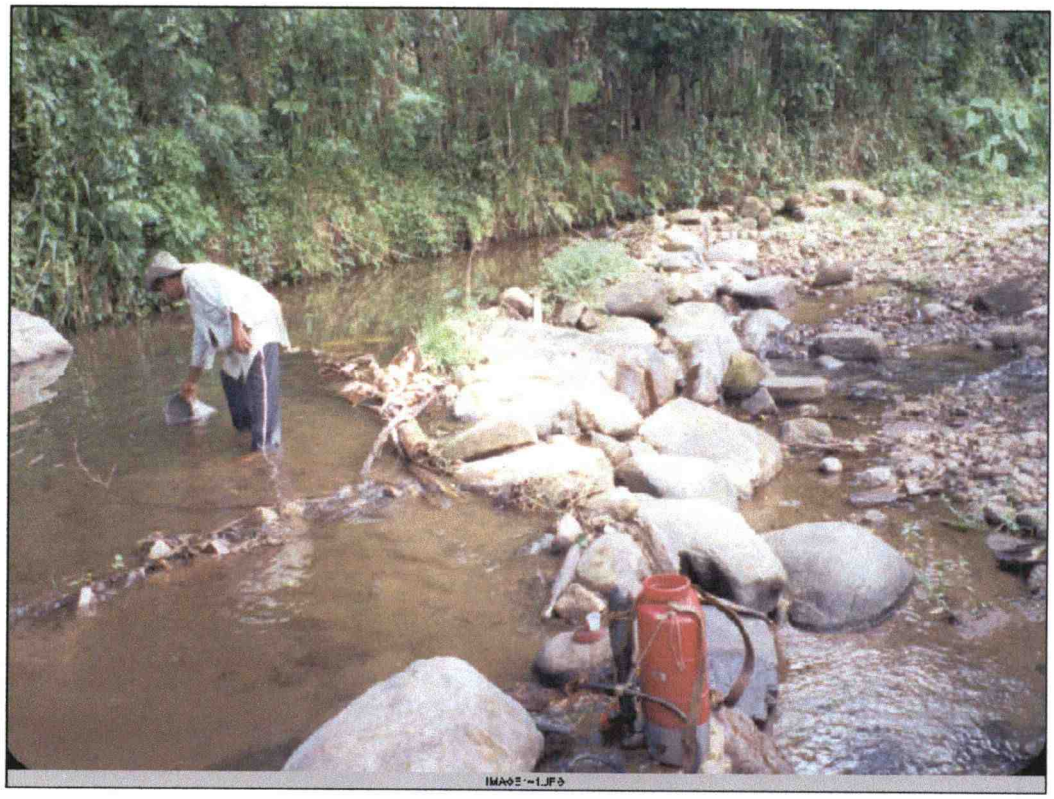


Figure 2.7a: Getting water from the stream for dilution

Figure 2.7b: Handling concentrate pesticide without gloves

Figure 2.7c: Applying pesticides with a leaking spray tank

Figure 2.7d: Spraying pesticides on an over-head canopy without a head protection, or facemask, and using minimal coverage.

Figure 2.7e: Ready to apply pesticides

Chromatograms of urine analysis for TCP: Chromatograms of pre and post application of chlorpyrifos of farmer number 4 is given in Figure 2.8(a-f) and a chromatogram of 0.25 µg/mL TCP standard is illustrated in Figures 2.9. Each sample run (method) was 25 min long and retention time of TCP-BSA was 12.47 min on a DB-1 column. A part of the chromatogram with interested peak is given as chromatograms in following figures.

Figure 2.8: Chromatograms for pre- and post-application urine extracts for farmer number three (a-f)

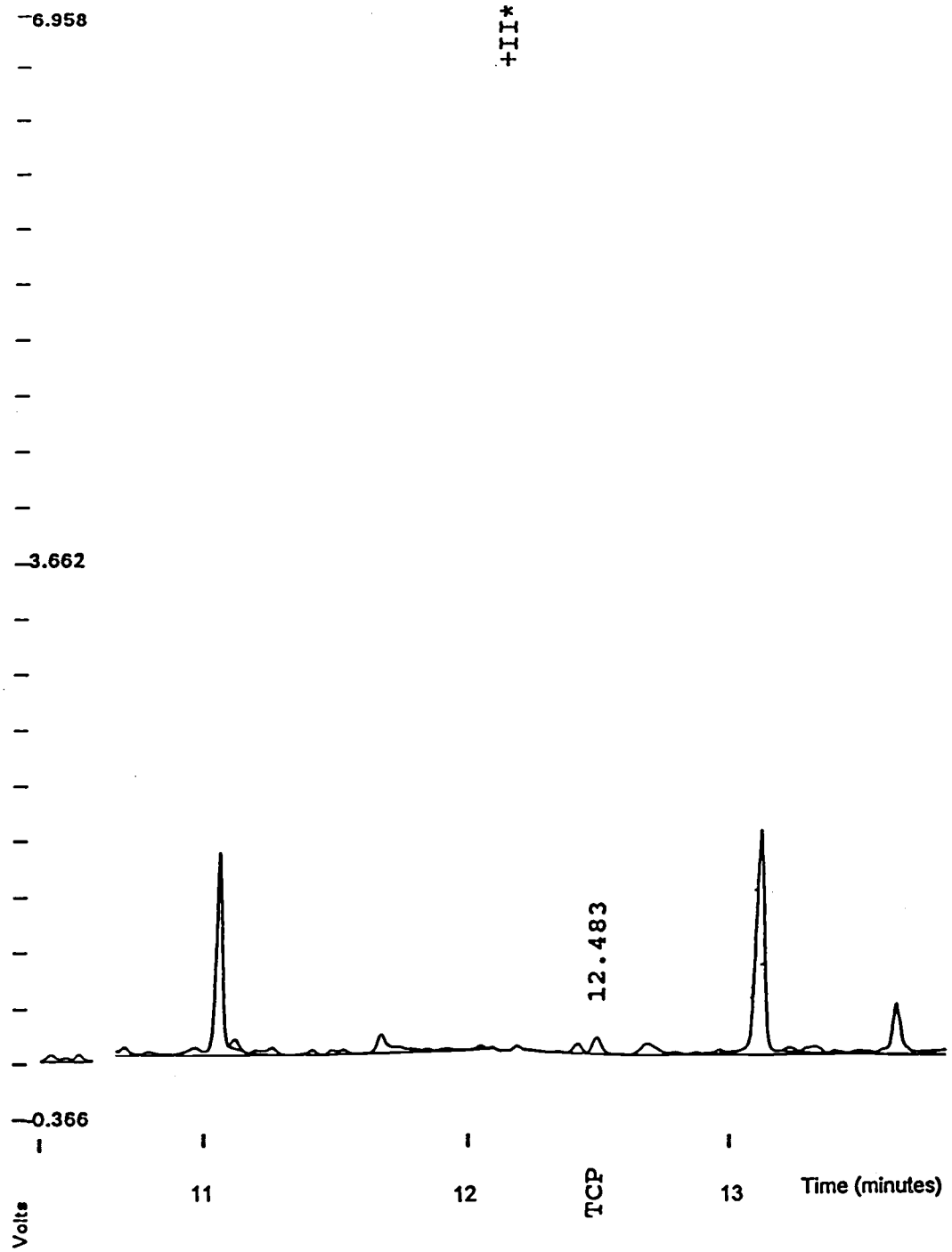


Figure 2.8-a: Chromatogram of pre-application urine extract of farmer number three (attenuation 3000)

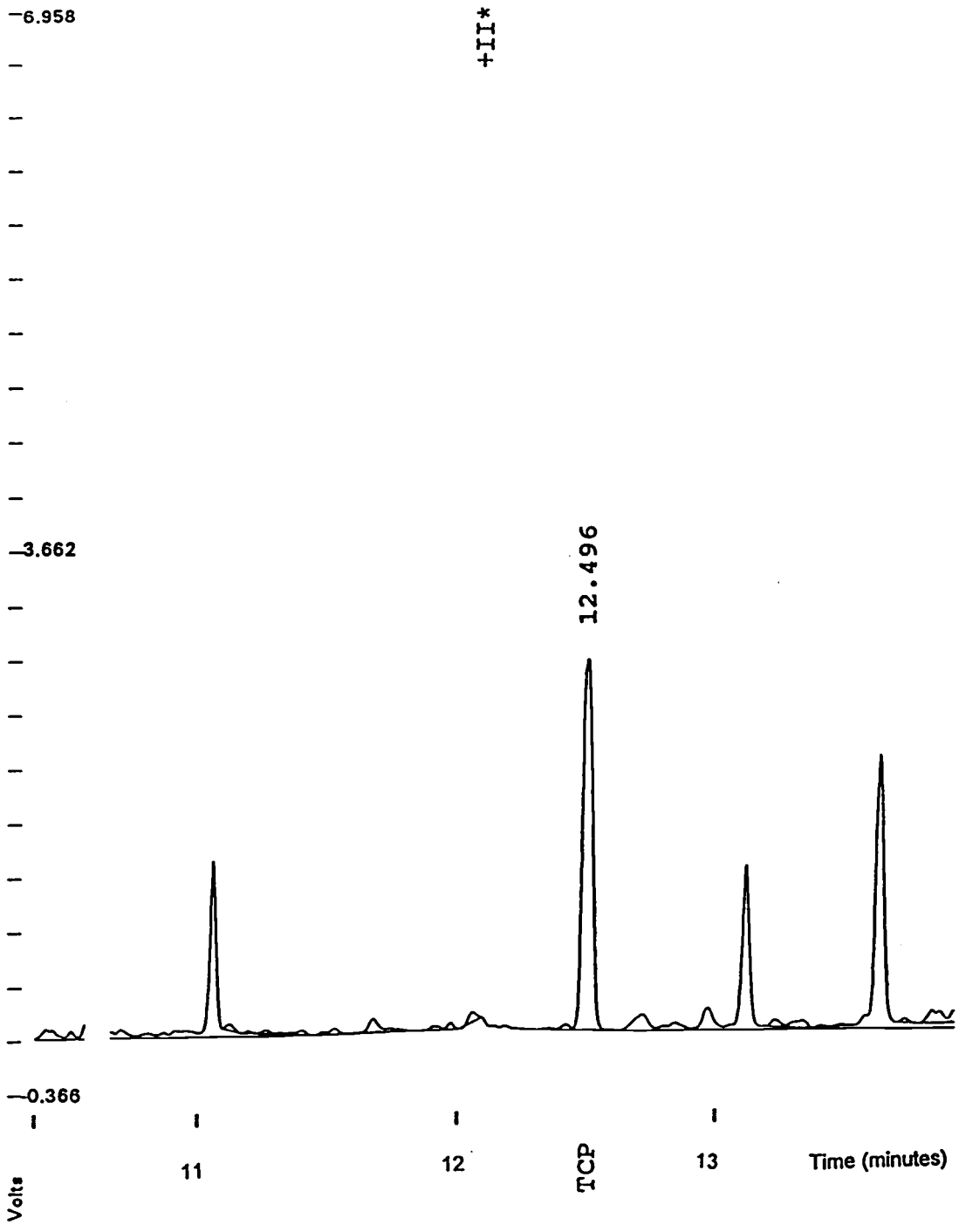


Figure 2.8-b: Chromatogram of 24 hr post-application urine extracts of farmer number three (attenuation 3000)

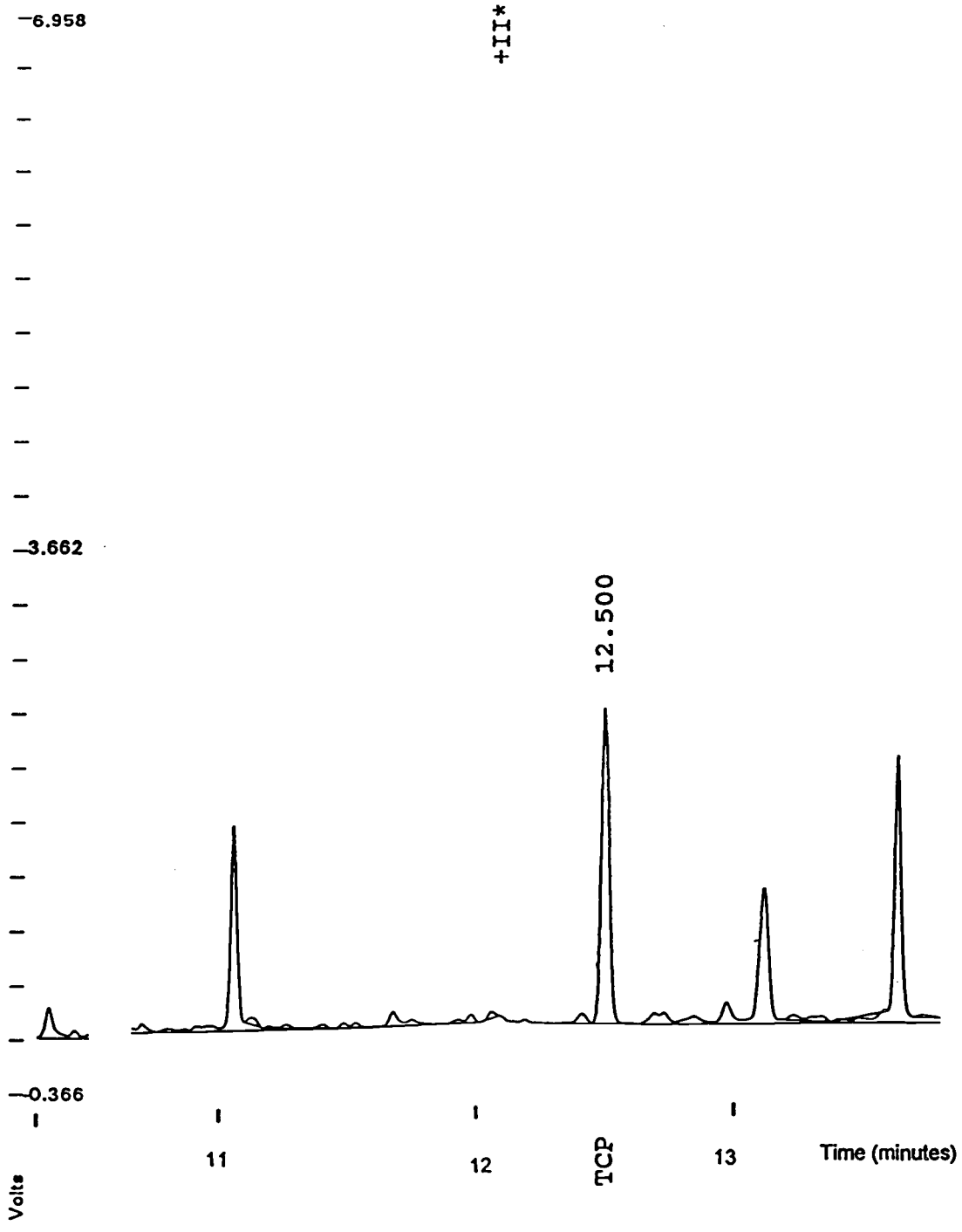


Figure 2.8-c: Chromatogram of 48 hr post application urine extract of farmer number three (attenuation 3000)

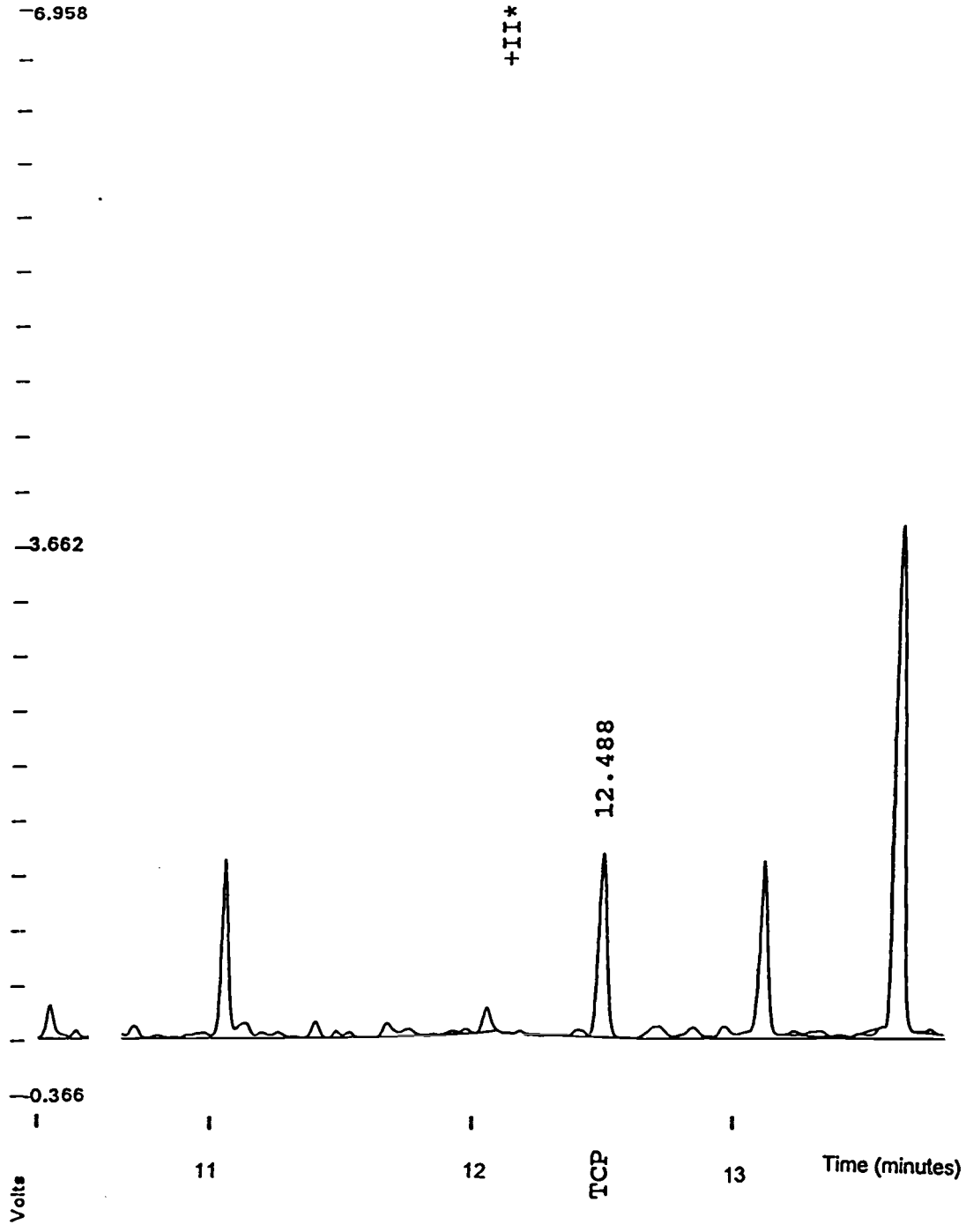


Figure 2.8-d: Chromatogram of 72hr post application urine extract of farmer number three (attenuation 3000)

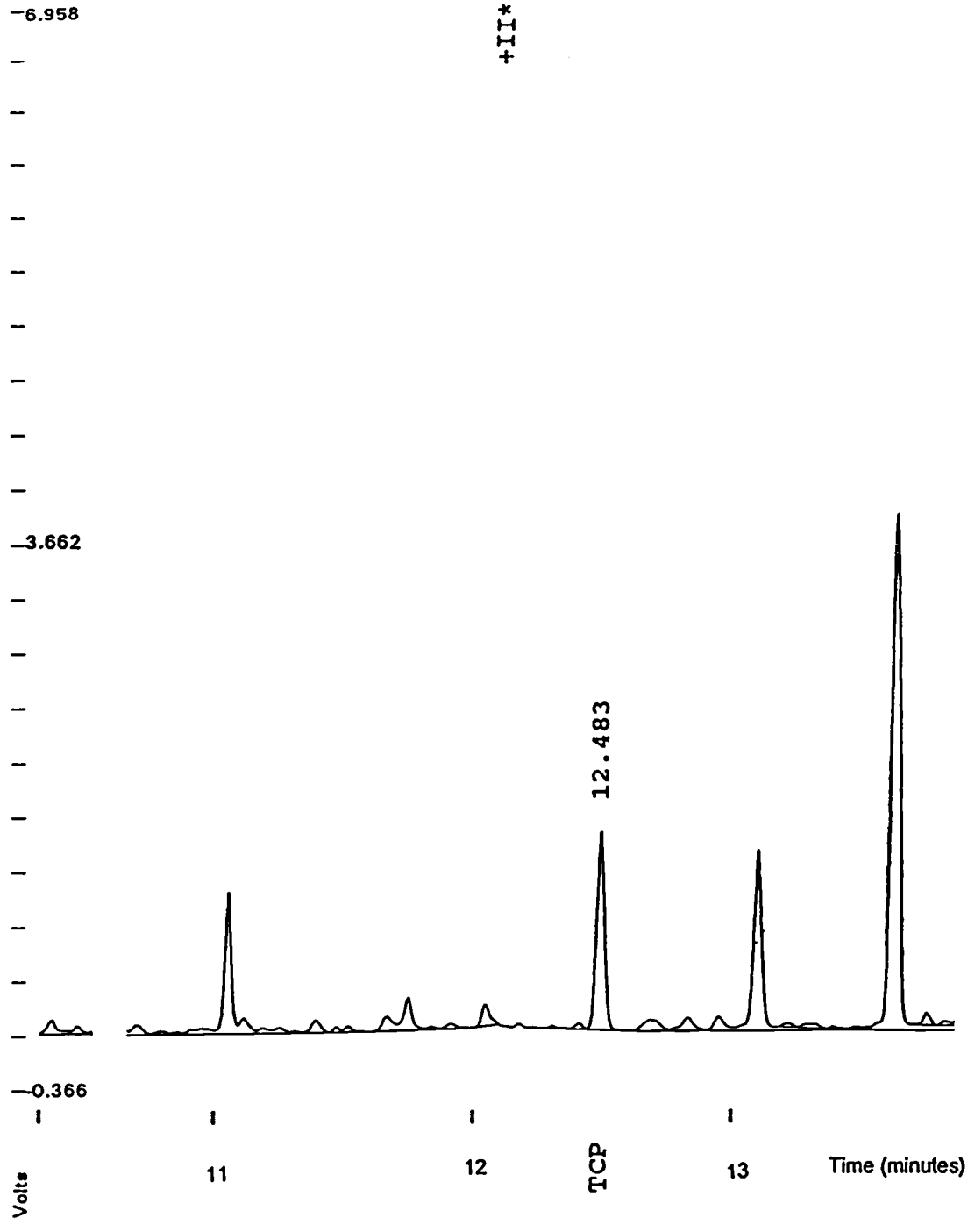


Figure 2.8-e: Chromatogram of 96 hr post application urine extract for farmer number three (attenuation 3000)

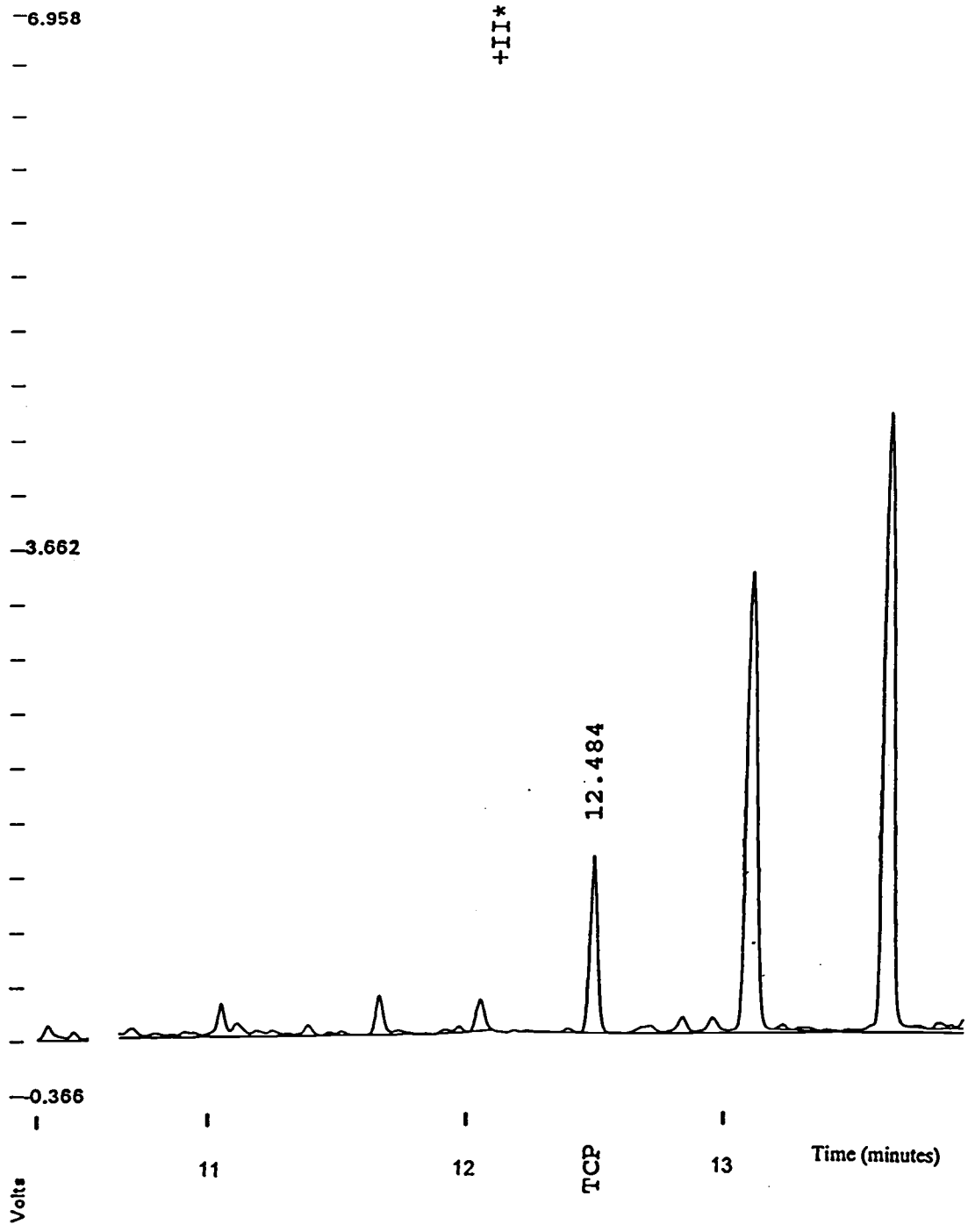


Figure 2.8-f: Chromatogram of 120 hr post application urine extract of farmer number three (attenuation 3000)

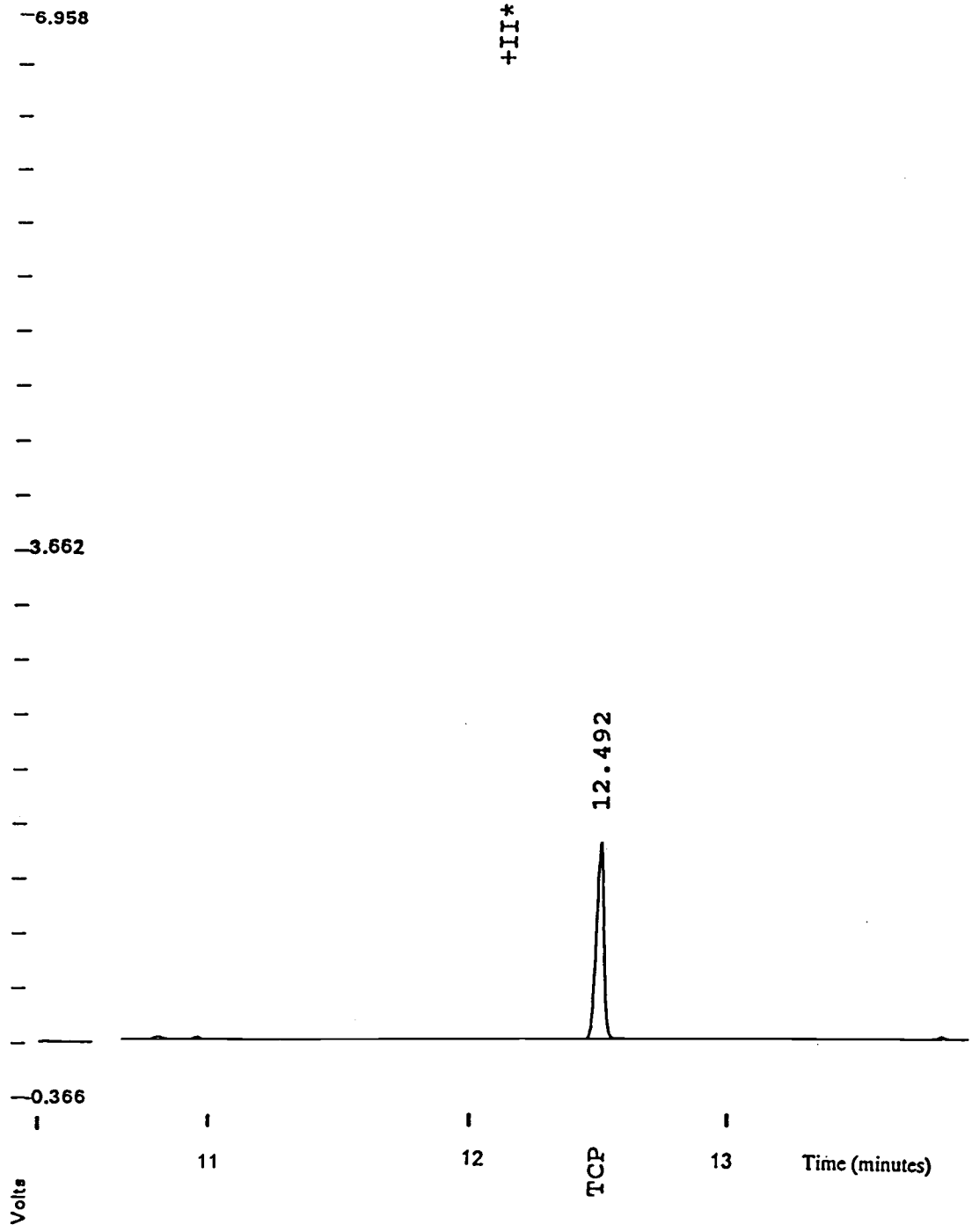


Figure 2.9: Chromatogram of 0.25µg/mL TCP standard (attenuation 3000)

Urinary TCP levels of individual farmers: Urinary TCP levels peaked 24hr after application and the levels dropped back to the baseline on the fifth day post application. The same pattern was observed for all farmers. Urinary TCP levels were expressed in μg/g of creatinine, assuming creatinine clearance of an adult is 1g per day. Average creatinine level was 0.95g/L for the farmers. Pre-(baseline) and post-application urinary TCP levels normalized for 1g of creatinine are given in Table 2.7. The values in Table 2.7 are derived from time verses TCP clearance curve for individual farmers, but not experimental values. Experimental values were obtained at 18, 42, 66, 90, and 114 hr post application and were extrapolated to 24 hr intervals using a polynomial curve and, as a result, some values obtained at 120 hr were negative. Total TCP excreted during the 5 day period ranged from 76.1-299.8 μg/5g of creatinine (mean 190.3 μg/5g of creatinine). All baseline samples except one had detectable levels of TCP, and baseline values were subtracted from all post application values for each farmer assuming this level was due to some continues exposure. Calculated cumulative TCP clearance values are given in Table 2.8. Since TCP elimination did not show exponential pattern, cumulative TCP clearance versus time graphs were used to calculate the time required to void half the amount of total TCP. Post-application urinary TCP levels with time for individual farmers and mean of all farmers with time are given in Figure 2.10(a-t) and 2.11, respectively.

Half time ($t_{1/2}$) : The time taken to eliminate 50% of the total TCP recovered in urine was considered as elimination $t_{1/2}$. The observed $t_{1/2}$ ranged from 24.8-35.1 days and the mean was 31.3 days.

Table 2.7: Pre- and post-application urinary TCP levels in μg/g of creatinine*

Farmer ID	Baseline	24 hour	48 hour	72 hour	96 hour	120 hour	Total (μg/5g of creatinine)
F1	0.02	83.6	53.3	30.5	7.7	-15.2	175.0
F2	43.57	92.9	60.4	36.3	12.2	-11.9	201.8
F3	5.04	35.1	24.8	16.5	8.2	-0.1	84.5
F4	3.94	60.0	39.8	28.5	17.2	5.9	151.4
F5	2.35	123.9	85.7	54.9	24.0	-6.9	288.6
F6	18.56	125.1	83.2	49.2	15.2	-18.8	272.7
F7	8.97	48.8	34.7	24.8	14.9	5.0	128.2
F8	5.90	116.3	86.7	54.5	22.4	-	279.9
F9	7.61	27.4	22.4	16.2	10.0	-	76.1
F10	2.84	115.4	83.4	58.5	33.6	8.8	299.8
F11	-6.04	89.3	64.8	47.0	29.3	11.5	241.9
F12	18.69	63.6	48.1	32.6	17.0	1.5	162.8
F13	8.52	96.8	75.7	48.8	22.0	-	243.3
F14	15.76	50.2	35.4	25.8	16.3	6.7	134.5
F15	14.56	119.4	82.2	51.3	20.4	-10.4	273.3
F16	9.59	58.9	41.3	24.2	7.2		131.6
F17	8.55	25.9	19.4	14.0	8.6	3.2	71.0
F18	12.16	90.8	66.6	47.0	27.4	7.8	239.7
F19	6.34	64.5	46.0	31.1	16.2	1.4	159.2
Mean	*9.84*	*78.31*	*55.46*	*36.40*	*17.35*	*-0.63*	*190.27*
Std.Devi	*10.28*	*33.02*	*23.05*	*14.46*	*7.66*	*8.62*	*75.92*
Std.Error	*2.36*	*7.57*	*5.28*	*3.32*	*1.76*	*2.03*	*17.41*

* These values are from the time versus TCP elimination curve (not experimental values)

Table 2.8: Post-application cumulative urinary TCP levels in μg/g of creatinine, calculated internal dose, and $t_{1/2}$

Farmer ID	24 hour	48 hour	72 hour	96 hour	120 hour	Internal dose mg/kg	$t_{1/2}$
F1	83.6	136.9	167.3	175.0	175	0.0053	24.8
F2	92.9	153.3	189.6	201.8	201	0.0057	26.3
F3	35.1	59.9	76.4	84.5	84.5	0.0023	30.4
F4	60.0	99.8	128.3	145.5	151.4	0.0044	33.5
F5	123.9	209.7	264.6	288.6	288.6	0.0080	29.3
F6	125.1	208.3	257.5	272.7	272.7	0.0084	26.1
F7	48.8	83.5	108.3	123.2	128.2	0.0040	36.0
F8	116.3	203.0	257.5	279.9	297.9	0.0082	29.2
F9	27.4	49.8	66.1	76.1	79.8	0.0021	34.0
F10	115.4	198.8	257.4	291.0	299.8	0.0075	33.2
F11	89.3	154.0	201.1	230.3	241.9	0.0067	35.1
F12	63.6	111.7	144.3	161.3	162.8	0.0051	32.3
F13	96.8	172.5	221.3	243.3	243.3	0.0070	30.6
F14	50.2	85.6	111.4	127.7	134.5	0.0037	34.9
F15	119.4	201.6	252.9	273.3	273.3	0.0080	28.4
F16	58.9	100.2	124.4	131.6	131.6	0.0038	27.4
F17	25.9	45.3	59.3	67.9	71.0	0.0023	37.6
F18	90.8	157.4	204.5	231.9	239.7	0.0077	33.8
F19	64.5	110.4	141.5	157.8	159.2	0.0050	31.8
Mean	*78.31*	*133.77*	*170.19*	*187.54*	*191.37*	*0.0055*	*31.29*
Std.Devi	*33.02*	*55.93*	*70.04*	*75.59*	*76.89*	*0.0022*	*3.65*
Std.Error	*7.57*	*12.83*	*16.07*	*17.34*	*17.64*	*0.00049*	*0.84*

Figure 2.10: Pre- and post-application urinary TCP levels of individual farmers (farmers number 1-19). X axis represent time in hr. Urinary TCP μg/g of creatinine is given in Y axis (Figures 2.10a-s)

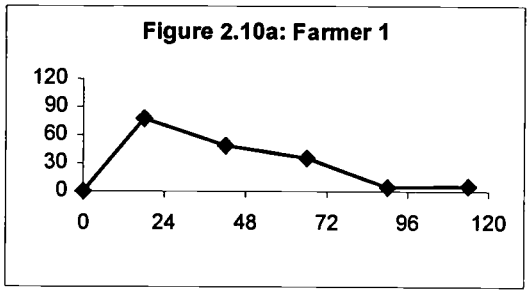


Figure 2.10a: Farmer 1
120
90
60
30
0
0
24
48
72
96
120

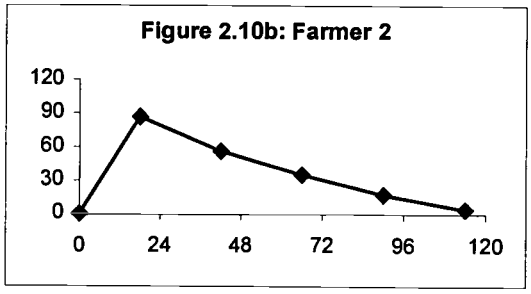


Figure 2.10b: Farmer 2
120
90
60
30
0
0
24
48
72
96
120

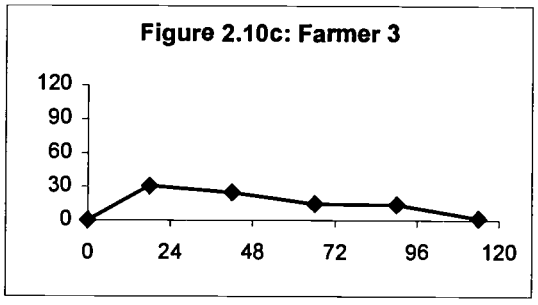


Figure 2.10c: Farmer 3
120
90
60
30
0
0
24
48
72
96
120

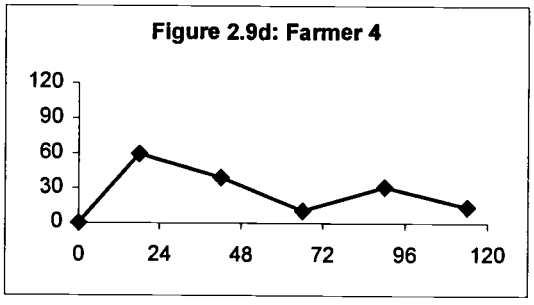


Figure 2.9d: Farmer 4
120
90
60
30
0
0
24
48
72
96
120

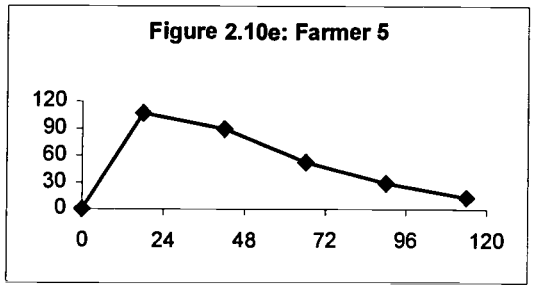


Figure 2.10e: Farmer 5
120
90
60
30
0
0
24
48
72
96
120

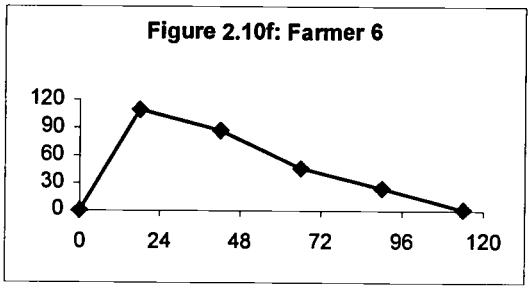


Figure 2.10f: Farmer 6
120
90
60
30
0
0
24
48
72
96
120

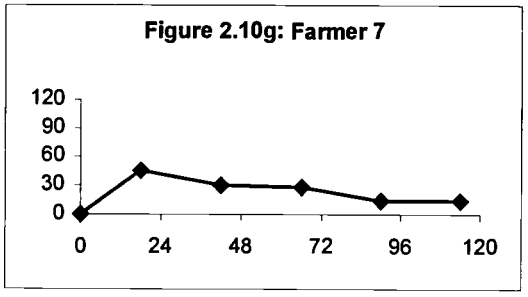


Figure 2.10g: Farmer 7
120
90
60
30
0
0
24
48
72
96
120

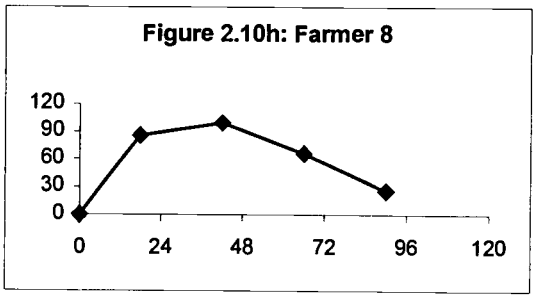


Figure 2.10h: Farmer 8
120
90
60
30
0
0
24
48
72
96
120

Figure 2.10i: Farmer 9

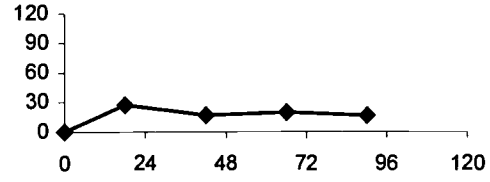


Figure 2.10j: Farmer 10

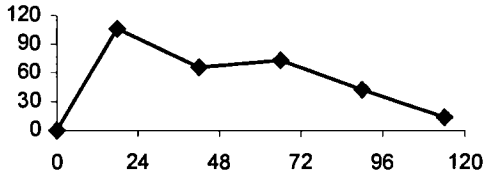


Figure 2.10k: Farmer 11

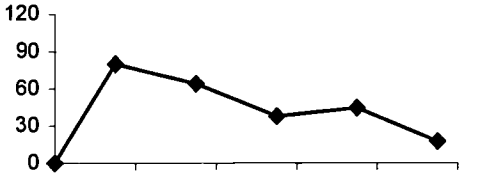


Figure 2.10l: Farmer 12

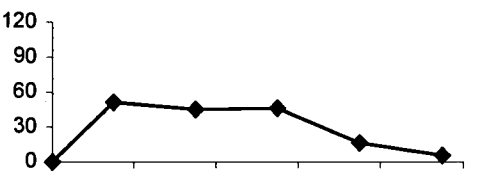


Figure 2.10m: Farmer 13

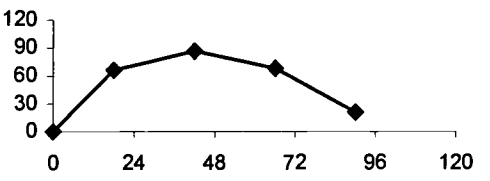


Figure 2.10n: Farmer 14

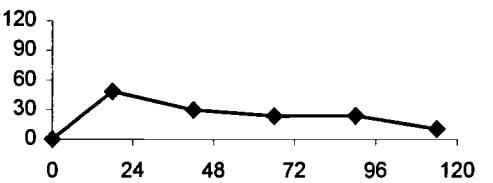


Figure 2.10o: Farmer 15

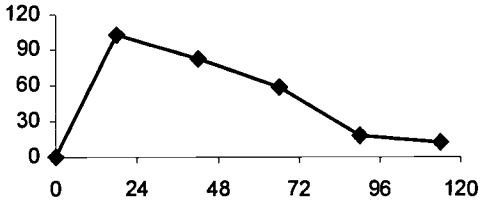
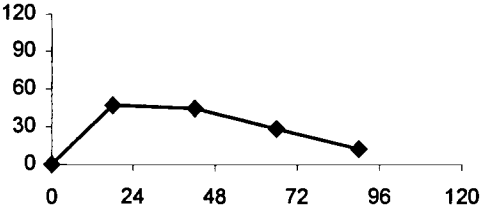


Figure 2.10p: Farmer 16



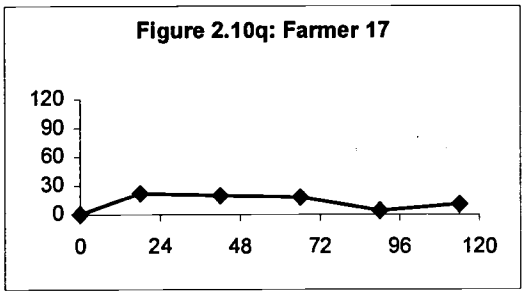


Figure 2.10q: Farmer 17
120
90
60
30
0
0
24
48
72
96
120

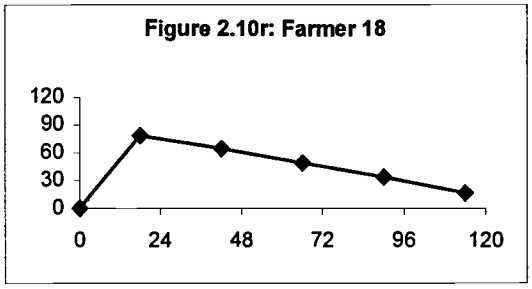


Figure 2.10r: Farmer 18
120
90
60
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24
48
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96
120

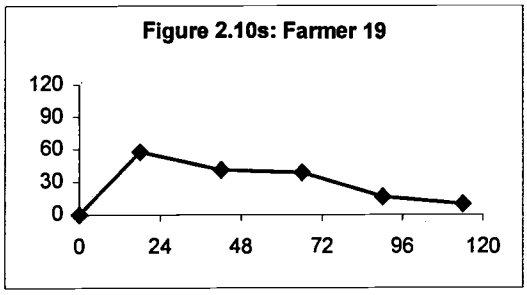
Figure 2.10s: Farmer 19
120
90
60
30
0
0
24
48
72
96
120

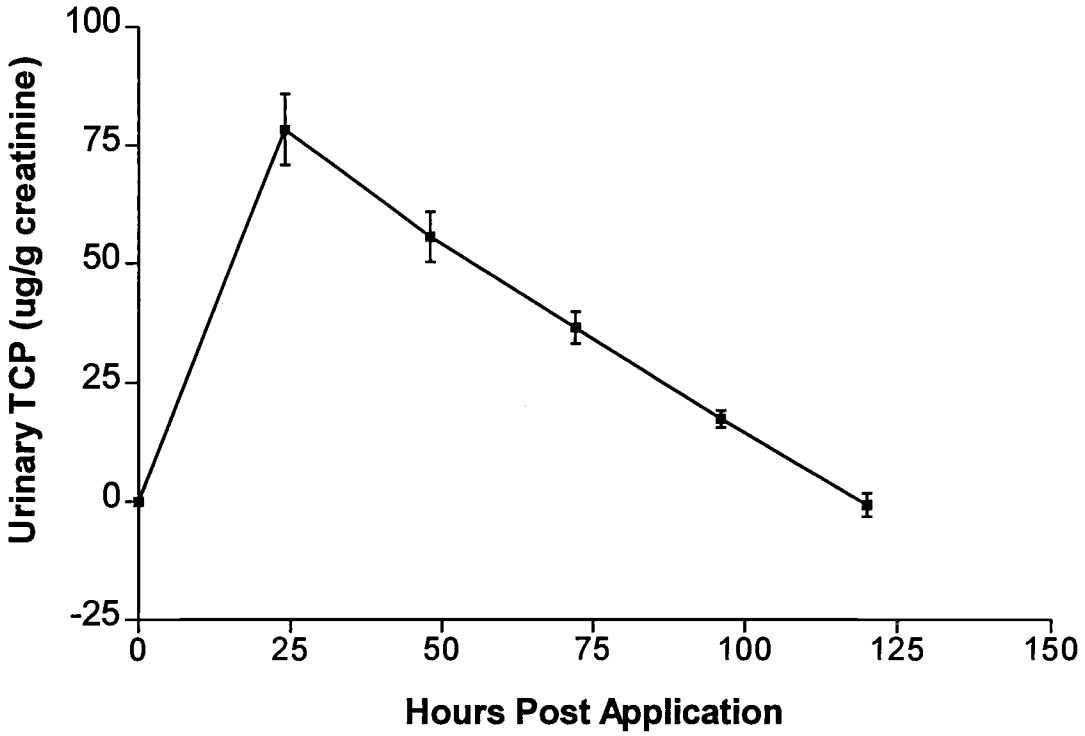

Figure 2.11: Mean urinary TCP levels of all farmers

Risk calculation: Based on Nolen et al. (1984) and Griffin et al. (1999), we assume 90% of the TCP was voided in urine over 5 days and one mole of chlorpyrifos generates one mole of TCP in the body.

Calculated total open skin surface areas for farmers are given in Table 2.9. Mosteller formula was used to calculate body surface area for each farmer using their body weight and height.

Amount of active ingredient used, body weight, calculated open skin area based on protective clothing used, dosage on skin, calculated internal dose, hazard quotient (HQ), and margin of safety (MOS) are given in Table 2.10. Griffin et al. (1999) reported no signs or symptoms of toxicity or change of erythrocyte or plasma cholinesterase levels with a dose of 28.59 mg over an area of 78 cm^2 on human skin. It was assumed that total internal dose was due to dermal exposure. Griffin et al. (1999) reported that 1% of the total dose was recovered after an 8hr exposure from the water based chlorpyrifos formulation applied on the skin. Therefore, proportional dermal dose was calculated for all farmers based on the duration of exposure and urinary TCP level. Duration of exposure was calculated from time started applying pesticide through the body-wash after application. Calculated internal dose values, HQ and MOS values ranged from 0.0021-0.0084 mg/kg, 0.8-2.7, and 3.6-14.3, respectively. EPA oral sub chronic NOEL and reference dose (RfD) used for the calculations was 0.03 and 0.003 mg/kg, respectively. An uncertainty factor of 10 was used for human variability to obtain the RfD (EPA, 1997). Equations used to calculate MOS and HQ are given below.

$$\text{Margin of Safety} = \frac{\text{NOEL (mg/kg body weight)}}{\text{Exposure Dose (mg/kg body weight)}}$$

$$\text{Hazard Quotient} = \frac{\text{Exposure Dose (mg/kg body weight)}}{\text{Reference Dose (mg/kg body weight)}}$$

All except three farmers showed an HQ higher than 1 (average 1.8), which indicates a risk to the applicator. The MOS values were greater than 1 in all cases. The farmers received an occupational dose higher than RfD of chlorpyrifos, but it was below the NOEL.

Medical Examination: A standard physical examination was conducted to assess possible adverse neurological effects of the farmers participating in this study. This examination evaluates the function of cranial nerves, muscle power, reflexes, co-ordination and sensations. No significant abnormalities were found in any of the farmers.

Table 2.9: Calculation of total body surface area and exposed area for farmers

Farmer ID	Total body surface area (m^2)	Percent exposed	Area exposed cm^2
F1	1.70	42.5	7225
F2	1.80	45.0	8100
F3	1.87	45.0	8415
F4	1.80	34.7	6246
F5	1.84	29.0	5336
F6	1.73	45.0	7785
F7	1.74	45.0	7830
F8	1.78	26.5	4717
F9	1.89	36.5	6899
F10	1.94	20.5	3977
F11	1.84	42.8	7875
F12	1.74	42.5	7395
F13	1.79	45.0	8055
F14	1.84	23.0	4232
F15	1.79	23.0	4117
F16	1.80	26.5	4770
F17	1.70	26.5	4505
F18	1.72	45.0	7740
F19	1.74	45.0	7830
Mean	1.790	36.26	6476.26
Std. Error	0.015	2.16	372.67
Std Dev.	0.066	9.42	1624.45

Calculation based on the Mosteller formula

Body Surface Area (m^2)= (Height (cm) x Weight (kg)/3600) ^{½}

Table 2.10: Calculated risk values for individual farmer

Farmer ID	Active Ingredient used (g)	Internal Dose of Chlorpyrifos mg/kg	Open skin area (cm^2)	Dose on Skin* mg/kg	$\mu g/cm^2$	HQ	MOS
F1	48.3	0.0053	4505	1.1	15.8	1.8	5.7
F2	72.5	0.0057	3690	1.0	19.6	1.9	5.3
F3	36.3	0.0023	7293	0.5	4.8	0.8	13.0
F4	48.3	0.0044	7326	0.7	6.8	1.5	6.8
F5	90.6	0.0080	5336	1.2	15.8	2.7	3.8
F6	90.6	0.0084	5017	1.2	15.5	2.8	3.6
F7	36.3	0.0040	7830	0.8	6.9	1.3	7.5
F8	60.4	0.0082	7565	1.2	10.6	2.7	3.7
F9	48.3	0.0021	3875	0.4	7.5	0.7	14.3
F10	90.6	0.0075	7081	1.1	12.3	2.5	4.0
F11	72.5	0.0067	6771	0.9	9.5	2.2	4.5
F12	48.3	0.0051	7395	0.9	8.0	1.7	5.9
F13	72.5	0.0070	5191	1.0	13.4	2.3	4.3
F14	48.3	0.0037	5336	0.6	8.1	1.2	8.1
F15	60.4	0.0080	8055	1.0	8.9	2.7	3.8
F16	36.3	0.0038	6570	0.9	9.7	1.3	7.9
F17	48.3	0.0023	3485	0.5	8.6	0.8	13.0
F18	60.4	0.0077	4988	1.1	14.0	2.6	3.9
F19	48.3	0.0050	7830	0.9	7.5	1.7	6.0

Assumption: 1) * Calculated assuming 1% of the dermal dose recovered in 8 hour period
2) Internal dose was used as the exposure and subchronic RfD for oral is 0.003 mg/kg

MOS= NOEL mg/kg/ Exposure mg/kg NOEL is 0.03mg/kg

Hazard Quotient = Exposed dose / RfD

STATISTICAL ANALYSIS

A statistical analysis was conducted to determine which of several application variables were associated with the internal dose of chlorpyrifos (μg/kg bodyweight). The variables included the amount of active ingredient (g) applied by the farmer and protective measures used which include condition of the spray tank (good, average and leaky) the protective clothing used, (short/long pants and/or short/long-sleeved shirt - see Table 2.11), and whether a hat and/or gloves were used. The duration of application was not considered since it was likely to be associated with the amount of pesticide applied, i.e., the more pesticide that is sprayed by a particular farmer (g), the longer the application period (hr). Also, since only one farmer wore a mask, an analysis of the significance of the use of a mask with dose was not possible.

The internal dose of chlorpyrifos for the 19 farmers participating in the study was computed from the total TCP (μg TCP/g creatinine per day) excreted in 5 days following exposure using the following formula assuming 90% of the internal dose is excreted in urine. Calculated internal doses are listed in table in Table 2.8.

Internal dose (μg/kg body weight) = 1111 x TCP(μg/g of creatinine)/Body weight (kg)

With a limited number of observations, the analysis was divided into two steps. The first analysis screened for existing interactions between: 1) The amount of active ingredient applied by the farmer, spray tank rating (good, average leaky), and total exposed skin surface area (cm^2). 2) The amount of active ingredient applied by the farmer (g chlorpyrifos) and the spray tank condition. The reason for substituting total exposed skin area in place of indicator variables for shirt, pants, gloves, and hat during the first analysis was to conserve degrees of freedom in the analysis for interaction. A weakness of the model, which will be overlooked for the time being, is that it assumes each

Table 2.11: Safety measures used while application of chlorpyrifos in the field

Farmer ID	Protective clothing used	Gloves		Tank		Hat		Total exposure points
		Yes/No	Exposure points	Condition	Exposure points	Yes/No	Exposure points	
F1	3		0	Leaking	1	H	1	5
F2	4		0	Good	5	F(cloth),H	2	11
F3	4		0	Good	5	-	0	9
F4	1	Used	2	Good	5	-	0	6
F5	3		0	Average	3	-	0	6
F6	3		0	Average	3	-	0	6
F7	1		0	Average	3	-	0	4
F8	1	Damaged	1	Leaking	1	H	1	4
F9	4	Used	2	Good	5	H	1	12
F10	2		0	Leaking	1	H	1	4
F11	2		0	Good	5	H	1	8
F12	1		0	Average	3	H	1	5
F13	3		0	Leaking	1	-	0	4
F14	3		0	Average	3	-	0	6
F15	1		0	Leaking	1	-	0	2
F16	2	Used	2	Average	3	H	1	6
F17	4		0	Good	5	H	1	10
F18	3		0	Leaking	1	-	0	4
F19	1		0	Average	3	-	0	4

1= Short sleeved shirt and short pans
2= Long sleeved shirt and short pans
3= Short sleeved shirt and long pans
4= Long sleeved shirt and long pans
H= A hat was used F= Face cover was used

area unit of exposed skin, regardless of location, will have a uniform degree of association with internal dose.

The second analysis, which would be conducted if no interactions were observed between spray tank or total exposed skin area and the amount of active ingredient, would be to explore an additive model computing internal dose associated with particular clothing worn by farmers (long-sleeved or short-sleeved shirt, long pants or short pants, hat, and gloves) after accounting for the amount active ingredient used and tank condition (assuming the latter two are significant factors).

Table 2.12: Analysis of Variance (ANOVA) results for interaction model: *Internal dose ~ a.i. + TANK + skin + a.i.:skin + a.i.:TANK*

Parameter	Degrees of Freedom	Sum of Squares	Mean Square	F Value	2-sided p-value (F) for terms added sequentially
a.i.	1	49.8659	49.8560	66.4731	0.0000047
TANK	2	16.5651	8.2825	11.3731	0.00210
Skin	1	4.1353	4.1353	5.6783	0.0363
a.i.:TANK	2	3.6300	1.8150	2.4923	0.128 (0.231)*
a.i.: skin	1	0.6171	0.6171	0.8474	0.377 (0.144)*
Residuals	13	8.0108	0.7283		

*p-value when a.i.:skin is added before a.i.:tank

RESULTS OF ANOVA:

The results in Table 2.12 indicate there is no evidence of interaction between the amount of active ingredient applied by the farmer and the condition of the spray tank, nor is interaction observed between exposed skin area and the amount of active ingredient applied. The results of a linear regression of the significant terms in the above model are listed in Table 2.13.

Table 2.13: Linear regression of model: *Internal dose ~ a.i. + TANK* + skin,* R^2=0.852

Internal Dose – (μg Chlorpyrifos/kg body weight) = β_0 + β_1·*a.i. (g)* +β_2·LEAKY_TANK +β_3·AVERAGE_TANK + β_4·*Skin*) d.f. = 14 (degrees of freedom), qt (0.975, 14) = 2.145

Parameter	**Coefficient value (β)**	**Std. Error (β)**	**95% u.c.l. (β)**	**95% l.c.l. (β)**	**p value (β) (2-sided)**
Intercept	-1.923	1.380	1.037	-4.883	0.185
a.i.	0.091	0.013	0.119	0.063	5.7×10^{-6}
AVERAGE_TANK	0.439	0.288	1.061	-0.183	0.150
LEAKY_TANK	0.502	0.151	0.828	0.176	0.005
.Skin	3.4×10^{-4}	1.6×10^{-4}	6.7×10^{-4}	2.1×10^{-6}	0.047

u.c.l.-upper confidence limit
l.c.l.-lower confidence limit

Internal Dose - (μg Chlorpyrifos/kg body weight) - computed from measured TCP levels in urine (μg TCP /g creatinine in urine)

a.i. - (g) Mass of active ingredient applied by farmer

LEAKY_TANK - indicator for leaking spray tank (1= leaky, 0=good or average)

AVERAGE_TANK - indicator for a spray tank rated as average (1=average, 0=good or leaky)

Skin (cm^2) - exposed skin area of the farmer during pesticide application.

*The categorical variable, *TANK*, consists of the indicator variables *LEAKY_TANK* and *AVERAGE_TANK*.

qt - t multiplier for 95 % confidence interval

SUMMARY OF STATISTICAL FINDINGS

Internal dose vs. active ingredient applied: There is overwhelming statistical evidence that the internal dose of chlorpyrifos increase with amount of active ingredient (a.i.) applied ($p< 6 \times 10^{-6}$; d.f.=14). An increase of 91 ng for the mean internal dose is associated with each additional gram of chlorpyrifos applied by the farmer (95% confidence interval is 63 ng/kg to 119 ng/kg increase per gram of active ingredient applied). The scope of inference of this model includes the 19 farmers studied and an application amount of chlorpyrifos between 36.3g and 90.6g.

Internal dose vs. spray tank condition: There is strong evidence (p=0.005) that an increase in internal dose is associated with the use of a leaky spray tank. A 502 ng chlorpyrifos/kg body weight increase in the mean dose is associated with farmers who used a leaky spray tank over farmers who used a spray tank in good condition (95% confidence interval is 151 ng/kg to 828 ng/kg). The model does not indicate a difference in internal dose between farmers who used tanks in either good or average condition (p=0.150). Farmers who used spray tanks rated as "average" were likely to have a mean increase in internal dose of 439 ng/kg body weight over farmers who used spray tanks rated in good condition; 95% confidence range 288 ng/kg *increase* to 183 ng/kg *decrease* in internal dose.

Internal dose vs. exposed skin area: An increase in internal dose is associated with increased exposed skin surface area (p=0.047). An internal dose increase of 0.339ng chlorpyrifos/kg body weight is associated with each additional square centimeter of exposed skin surface area (95% confidence interval 0.156 to 0.677ng chlorpyrifos/kg body weight). According to Table 2.10, the farmers had exposed skin areas ranging from 3485 cm^2 to 8055 cm^2 with an average of 6060 cm^2. This corresponds to an increase in internal dose

between 1.18 to 2.73 μg chlorpyrifos/kg body weight with an average of 2.05 μg/kg associated with exposed skin area.

Since no interaction was observed between any variables, a second analysis (an additive model) was conducted to explore the degree to which internal dose could be associated with the protective garments worn by the farmer (see Figure 2.4 and Table 2.8). A full model was constructed using categorical variables for tank condition (good, average, and leaking), whether the farmer wore a short-sleeved shirt or long-sleeved shirt; short pants or long pants. Numerical variables in the model included the amount of active ingredient, a.i. (g), applied by the farmer and the number of gloves worn by the farmer.

The full model was then subject to a stepwise regression to determine which parameters were significant. Table 2.14 lists the Analysis of Variance (ANOVA) results of the selected model: *Internal Dose ~ a.i. + LONG_SHIRT + LONG_PANTS + TANK.*

Table 2.14: ANOVA results for model selected from step-wise regression procedure

Parameter	d.f.	Sum of squares	Mean square	F Value	2-sided p-value (F) for terms added sequentially
a.i.	1	49.9865	49.9865	90.6307	3.17×10^{-7}
TANK*	2	16.5651	8.2825	15.0534	0.000413
SHIRT	1	5.8497	5.8497	10.6317	0.006200
PANTS	1	3.3908	3.3908	6.1627	0.027482
Residuals	13	7.1527	0.5502		

d.f.- degrees of freedom

*The categorical variable, *TANK,* consists of the indicator variables *LEAKY_TANK* and *AVERAGE_TANK.*

Full Model: (Upper case variables correspond to factor, i.e. 1 or 0; lower case are numerical)
Internal Dose ~ a.i. + LONG_SHIRT + LONG_PANTS + LEAKY_TANK + AVERAGE_TANK + hat + gloves

Table: 2.15: Results of a linear regression of the stepwise regression from the full model: Internal Dose (μg chlorpyrifos/kg body weight) ~ a.i. + LEAKY_TANK + AVERAGE_TANK + LONG_SHIRT + LONG_PANTS,
$R^2 = 0.914$

Internal Dose – (μg chlorpyrifos/kg body weight) = β_0 + β_1·*a.i. (g)* +β_2·LEAKY_TANK +β_3·AVERAGE_TANK + β_4·LONG_SHIRT + β_5· LONG_PANTS , n = 19 (number of farmers) d.f. = 13 (degrees of freedom), qt (0.975, 13) = 2.160

Parameter	**Coefficient value (β)**	**Std. error (β)**	**95% u.c.l. (β)**	**95% l.c.l. (β)**	**p value (β) (2-sided)**
Intercept	-0.094	0.6232	1.2521	-1.4401	0.8823
a.i.	0.092	0.0101	0.1138	0.0702	5.47×10^{-7}
LEAKY_TANK	0.420	0.1226	0.6848	0.1552	0.00452
AVERAGE_ TANK	-0.173	0.3054	-0.4866	-0.8327	0.58145
LONG_SHIRT	-0.857	0.2485	-0.3202	-1.3938	0.00453
LONG_PANTS	0.442	0.1784	0.8273	0.0567	0.02748

u.c.l. - upper confidence limit

l.c.l. - lower confidence limit

Internal Dose - (μg Chlorpyrifos/kg body weight) - computed from measured TCP levels in urine (μg TCP /g creatinine in urine)

a.i.- (g) Mass of active ingredient applied by farmer

LONG_SHIRT - indicator for whether farmer wore a long or short-sleeved shirt (1= long-sleeved, 0= short-sleeved)

LONG_PANTS - indicator for whether farmer wore long or short pants (1=long pants, 0= short pants)

LEAKY_TANK - indicator for leaking spray tank (1= leaky, 0=good or average)

AVERAGE_TANK - indicator for a spray tank rated as average (1=average, 0=good or leaky)

hat - numerical variable for the degree of facial protection used (0=no hat used, 1=hat used, 2=hat + mask used)

gloves - numerical variable for the number of gloves worn by the farmer during application, 0=no gloves, 1=one glove, and 2= two gloves worn.

SUMMARY OF STATISTICAL FINDINGS

Internal dose vs. active ingredient applied: There is overwhelming statistical evidence that computed internal dose levels of chlorpyrifos from measured levels of TCP in urine for the nineteen farmers are associated with amount of active ingredient (a.i.) applied ($p< 5 \times 10^{-7}$;d.f.=13). Similar results were found in the previous linear regression model. The mean internal dose level increases by 92ng Chlorpyrifos/kg body weight per gram of chlorpyrifos applied by the farmer (95% confidence interval is 70 μg/kg to 114 μg/kg increase per gram of active ingredient applied). These results are in agreement with the previous analysis in Table 2.13.

Internal dose vs. spray tank condition: Both full model and liner regression model had strong evidence (p=0.005) that the use of a leaky spray tank corresponds to an internal dose increase of 420ng Chlorpyrifos/kg body weight, over farmers who used a spray tank rated in good condition (95% confidence interval is 155 ng/kg to 685 ng/kg). The model does not indicate a difference in internal dose between farmers who used spray tanks evaluated in either good or average condition (p=0.581). The *decrease* in dose associated with the use of a spray tank evaluated in average condition is 173 ng/kg body weight over farmers who used a spray tank in good condition; 95% confidence

range 581 ng/kg *increase* to 832 ng/kg *decrease* in internal dose. These results are also in agreement with the previous analysis in Table 2.13.

Internal dose vs. clothing: There is strong evidence that a decrease of internal dose is associated with the use of a long-sleeved shirt instead of a short-sleeved shirt (p=0.004). A mean *decrease* in internal dose of 857 ng Chlorpyrifos per kilogram bodyweight was observed for the farmers who wore a long-sleeved shirt over those who wore short-sleeved shirts; 95% confidence interval, 320ng/kg to 1,39 ng/kg decrease.

Since the parameters for GLOVES and HAT were eliminated in the stepwise regression, it is concluded that there is no statistical evidence that a change in additive internal dose for the farmers is associated with the use of gloves or a hat.

SCOPE OF INFERENCE

The scope of inference includes the 19 farmers having characteristics, using application methods and following safety measures listed in Tables 2.8 - 2.11. Table 2.16 summarizes the contribution of each factor towards the internal dose of a farmer representative of the average of the body weight, computed background internal dose, and amount of active ingredient applied. The average contribution of each factor is computed from the coefficient values in Table 2.15 multiplied by the average value of the factor.

Table 2.16: Additive contributions of each significant factor to computed Internal Dose (μg/kg) of the average farmer studied, using results in Table 2.15

Model: *Internal Dose~ Background + a.i. + Leaking Tank + Short Shirt + Long Pants*

Factor	Avg. contribution of factor to internal dose (ug/kg)	Cumulative internal dose (ug/kg)	% Total internal dose
Active ingredient applied (Avg. = 58.82 g)	5.41	5.67	76%
Leaking Spray tank	0.42	6.09	6%
Short-sleeved shirt	0.86	6.95	12%
Long Pants	0.44	7.39	6%
Total Internal Dose (μg/kg)	7.13		
Total Dose (μg) for Avg. body wt. (69.3 kg)	512.46		

Avg. =Average

DISCUSSION

Consistent TCP excretion patterns were observed in all 19 farmers. The average TCP excretion half-life of 31.3 days was consistent with a 30 day half-life reported, by Griffin for dermal exposure of alkylphosphates. With a 1:1 relation with diethyl thiophosphate and diethyl phosphate one might expect a comparable half life from the two metabolites. Griffin also reported a 15hr half-life from oral exposure to chlorpyrifos. One can conclude that the farmers exposure to chlorpyrifos is dermal rather than respiratory. Backpack sprayers would find to provide larger droplets rather than the fine aerosols that could enhance respiratory uptake. Structural applicators working in confined areas show only 26% of their exposure coming through the respiratory route.

The use of the EPA reference dose of 0.003 mg/kg/day is not as appropriate toxicological reference for this exposure scenario. The RfD is based on a sub-chronic study in human were exposed to 19 days (0.03 mg/kg/day) with plasma acetyl cholinesterase monitored as the response. While the later enzyme is sensitive to chlorpyrifos it is not a reliable indicator of adverse effect. Aslo farmers are experiencing a one time exposure. A better reference is the toxico-kinetic study by Griffin who did not observed a cholinesterase inhibition with dermal dose of 28.59 mg. The maximal exposure received by the farmer was 29.9 mg, which is almost same as dermal exposure study. On this basis, the farmers experience a minimal risk despite taking limited precautions.

There has been concern that farmers in tropical regions are particularly vulnerable because of the reluctance to use protective clothing under the hot and humid climatic conditions that are common in these regions. This study demonstrates that the farmers risk can be minimize by limiting the amount applied and the frequency of applications. It should also be noted that the

study deals with a worst-case scenario where the farmer is spray an overhead canopy.

With observations on 19 farmers and variation in the internal dose experienced a statistical analysis provided perspective on the effects of different variables influencing exposure. It is clear that the amount of compound applied is the over siding factor. However, the use of sound equipment and long-sleeved shirt can reduce exposure by 6-10%. The observation that wearing long pants actually increased exposure was surprising and would need to be confirmed. However, the farmer may not wash his legs after application when wearing long pants and it he were to continue to wear these pants his exposure could be prolonged.

CONCLUSION

Farmers applying chlorpyrifos showed a consistent excretion pattern of the metabolite, TCP, characteristic for this organophosphate. The excretion half-life ranged from 24.8 to 37.6hr with an average value 31.3hr. The cumulative TCP excreted over 120hr was used to calculate the internal dose of chlorpyrifos, which ranged from 0.0021 to 0.0084 mg/kg. It was assumed that major exposure route was skin, and a dose of 0.4 to 1.2 mg/kg was estimated, based on 1% dermal uptake. This dose was considered to give a marginal risk with hazard quotients range from 0.7 to 2.7 and margin of safety from 4 to 14. Statistical analysis established that the internal dose was determined, in large part, by the amount of chemical applied. In addition, it was demonstrated that faulty spray equipment and the amount of skin exposed also was associated with an increase in the internal dose. Analysis also indicated that wearing long pants could increase the internal dose, although the reason for this unexpected response is not clear. This study provides quantitative information for that program, which can be used to train farmers in the use of safer application practices.

CHAPTER 3

ANALYSIS OF DRINKING WATER AND HOUSE DUST FOR CHLORPYRIFOS AND 3,5,6-TRICHLORO-2-PYRIDINOL, COLLECTED FROM A FARMING COMMUNITY IN SRI LANKA

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ABSTRACT

Drinking water was collected from three wells and dust was collected from three houses located in a major vegetable growing area in Kandy district. Wells and houses were located adjacent to the cultivated land, some of which had been treated with chlorpyrifos. Water samples were analyzed for chlorpyrifos and the major metabolite, 3,5,6-trichloro-2-pyridinol (TCP). Floor wipes were analyzed for possible contamination by the parent compound. Chlorpyrifos in drinking water was below quantifiable level, but 9, 10, and 0.6 ng/mL of TCP were detected in well water samples. In the dust analysis, quantifiable peaks were found in the same window as chlorpyrifos, but the results were not confirmed on a second column. Recoveries of 94% and 86.8% of chlorpyrifos and TCP from water were achieved with detection limit of 13 ng/L, and 18ng/mL, respectively. Recovery of the parent compound from spiked dust was 72% with a detection limit of 167 ng/L.

Prevailing climatic conditions favor dissipation of chlorpyrifos from water and soil, limiting the risk of chlorpyrifos exposure from these sources.

INTRODUCTION

Chlorpyrifos is used widely on soil and crops to control insect pests on farm animals, to control ticks, and in houses to control cockroaches, fleas, and termites. The manufacturer voluntarily withdrew chlorpyrifos from most indoor and pet uses in 1997 (United Stated Department of Health and Human Services, 1997). Chlorpyrifos neither bio-accumulates nor persists in the environment for extended periods.

Soil level of chlorpyrifos depends mainly on the amount applied and the disposal of waste containers in soil. Much of the compound applied to foliage eventually reaches soil, either as parent compound or metabolite (Racke, 1993). Re-deposition of atmospheric chlorpyrifos (Racke, 1992) and spills during storage, transportation, mixing, or cleaning of spray equipment could also contribute to soil levels of chlorpyrifos. Environmental factors such as moisture, pH, and organic carbon can greatly influence the fate of chlorpyrifos in soil (Harmaker et al., 1972; Getzin, 1981a,b,; Chapman and Chapman, 1986). Chlorpyrifos undergoes hydrolysis and microbial degradation in soil. The rate of hydrolysis is pH and temperature dependant (Miller and Zepp, 1983). The half-life was shorter in natural soils than in sterile soils, which illustrates the role of microbes. Under laboratory conditions, chlorpyrifos degradation half-life varies from less than 10 days to greater than 120 days in different soils (Meikle and Hedlund, 1973; Davis and Kuhr, 1976). The primary hydrolysis product, 3,5,6-trichloro-2-pyridonil (TCP), and secondary metabolite, 3,5,6-trichloro-2-methoxy pyridine will mineralize to CO_2 (Bidlack, 1979; Chapman and Harris, 1980; Getzin, 1981a; Racke et al., 1988). The fate of chlorpyrifos in the environment is illustrated in Figure 1.5.

Racke et al. (1990) evaluated the potential for enhanced microbial degradation of chlorpyrifos in different soils under laboratory conditions. Repeated chlorpyrifos applications to soils did not alter the rate of degradation or product distribution. The reported half-life of chlorpyrifos was 4-9 days in

soils with a pH greater than 8 and repeated applications of insecticides failed to control target pests. They concluded that chlorpyrifos is not susceptible to enhanced microbial degradation and repeated applications did not have any increased effect on the efficacy or persistence due to higher rate of metabolism. This was explained by the fact that the hydrolysis step was not due to microbial activity. Accumulation or mineralization of TCP was unrelated to the rate of chlorpyrifos hydrolysis, which was a function of microbial activity.

Chlorpyrifos has an average sorption coefficient (Koc) of 8500 mL/g (Recke, 1993) and will tend to sorb in soil; hence, there is less potential to leach from soil in solution. While chlorpyrifos has been considered immobile in soil (Racke et al., 1993), TCP is moderately mobile due to its greater water solubility. Chlorpyrifos may degrade by photo-induced reactions on the soil surface. Laboratory studies using UV light (254nm from mercury lamp) demonstrated that photochemical processes such as hydrolysis, dechlorination, and oxidation take place simultaneously (Walia et al., 1988). Dehalogenated and oxidized products undergo further photolysis to form chloropyridinol and O,O-deethyl phosphorothioic acid. In the same study, the levels of these metabolites was also decreased with time, suggesting mineralization taking place under UV-photo-irradiation conditions.

In water, partition into colloids, evaporation, hydrolysis, and photosensitized oxidation are likely to be the major pathways of dissipation. Distilled water with pH 1 or 12.9 had a half-life of 89 or 0.01 days, respectively, at 25 ^{0}C (Macalady and Wolfe, 1983). In a similar study, Freed et al. (1979) reported a half-life of 120 and 53 day at pH of 6.1 and 7.4 at 20 ^{0}C. The activation energy for the hydrolysis of chlorpyrifos at pH 7.4 is 14 kcal/mol, indicating its sensitivity to temperature change. Hydrolysis can be catalyzed by copper ions (Blanchet and St. George, 1982). Henry's law constant (H) for chlorpyrifos is 6.6×10^{-6} atm-m3/mol (Downey, 1987), and the vapor pressure is 1.9×10^{-5} mmHg at 25 ^{0}C (Racke, 1993). Compounds with H of less than 10^{-5}

atm-m3/mol may volatilize slowly from water (Lyman et al., 1990), but it will also partition into available airborne particulate (Eisenreich et al., 1981).

In an exposure assessment study performed for residential environments in Arizona, Sydney et al. (1999) reported that chlorpyrifos level in indoor air was 3.3μg/m^3. The range of chlorpyrifos levels found in floor wipes and windowsill wipes was 0.004-48.5 and 0.07-16100 ng/m^2, respectively.

A farm worker might be exposed to chlorpyrifos during mixing and application or by consuming contaminated foods or water. Little children walking or crawling on contaminated house floors are also susceptible to exposure. US Environmental Protection Agency (US EPA) recommends a 24hr waiting period prior to reentering a chlorpyrifos-treated field. Chlorpyrifos has been found in at least seven current and former EPA National Priority List (NPL) hazardous waste sites (HazDat, 1996) and, thus, the potential for chlorpyrifos exposure is significant.

OBJECTIVES

A prior study evaluated occupational exposure to chlorpyrifos by analyzing urinary metabolites. The overall analysis of risk should consider other possible routes of exposure. Since wells used for drinking water were located in/or adjacent to treated areas and dust could be blown or tracked into homes. Therefore, the objective of this study is to analyze drinking water and house dust for the parent compound and the major metabolite to assess potential background exposure to chlorpyrifos. The study was carried out at the same site (Kandy district of Sri Lanka) as the prior experiment (Figure 3.1).

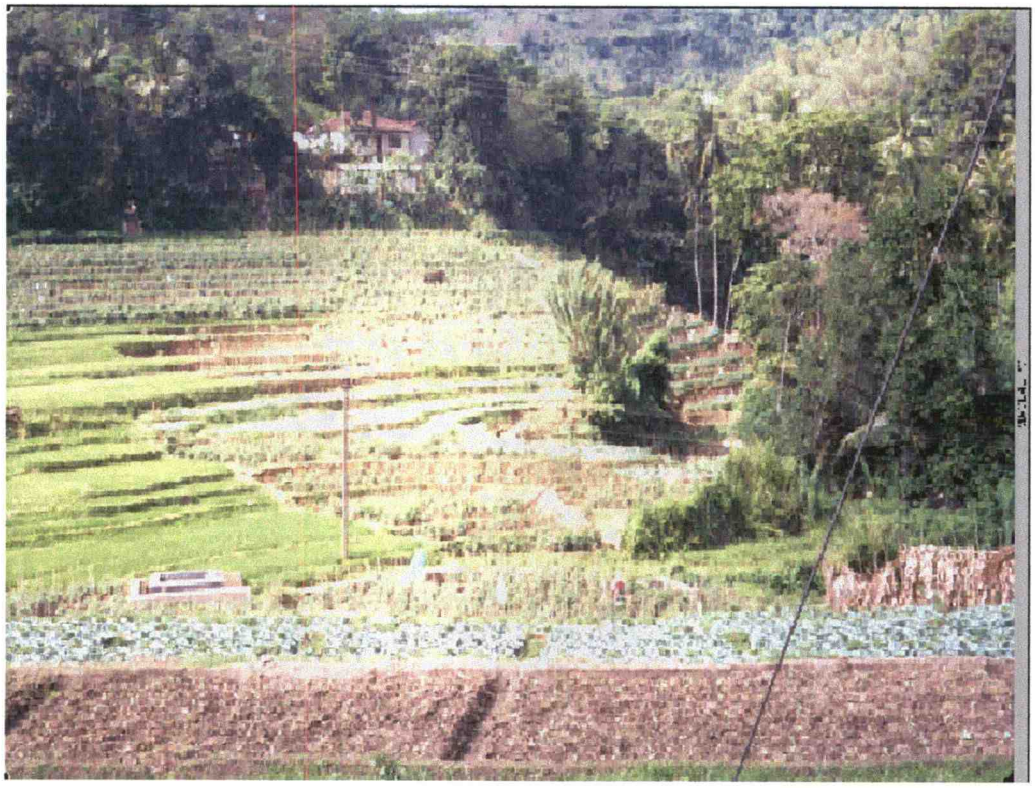

Figure 3.1: Location of drinking water wells and houses in the selected agricultural site

METHOD

Solvents and standards: Acetone, methylene chloride, ethyl acetate, hexane, and methanol were from Fisher Scientific, New Jersey. Chlorpyrifos and 3,5,6-trichloro-2-pyridinol standards are kind donations from Dow Agroscience, Indianapolis. All glassware was baked for 10hr at 350 ^{0}C before use.

Sample collection: Water samples were collected (about 3 weeks after the season) from three drinking water wells located in the selected farming community in Kandy district, Sri Lanka. This area uses contour landscaping, and crops are grown on contour plots. Houses are located at higher elevations around the field, and water wells are in the center, close to the lowest point, where the water level is near the ground water table (Figure 3.2). Samples were collected from three drinking water wells (three 1L samples from each well) at the end of the season. Bottles made with polyethylene terepthalate manufactured by CISCO Specialty Packaging (Pvt.) Ltd., Pannipitiya, Sri Lanka were used. Water pH was adjusted to 2 as specified in the EPA method 525.2 (USEPA, 1994) to minimize both hydrolysis and microbial activity. Samples were refrigerated until used.

Dust was collected from three houses located in the same farming community. Two out of three houses were facing the cultivated field and the other house was about 100m away. Dust was collected using cotton balls from an area of 2ft^2 from 3 locations of the house, i.e., front porch, living room and kitchen (two replicates from each locations). Samples were stored in five mL glass vials and kept refrigerated until analyzed. Both water and dust were brought to the Food Safety and Environmental Stewardship Program Laboratory at Oregon State University for extraction and analysis.

Figure 3.2: Location of a drinking water well and sampling

ANALYSIS OF DRINKING WATER FOR CHLORPYRIFOS

A sub-sample of 200 mL was extracted with hexane (2 x 10 mL aliquots) in separatory funnels. Extracts were combined and the volume adjusted to 20 mL using weight and density of hexane at room temperature. An aliquot of 10 mL from the total volume was concentrated to 1 mL prior to analysis. A varian gas chromatograph system fitted with an electron capture detector was used and 2 μL injections were made using the auto-sampler.

Recovery of chlorpyrifos from spiked water: Deionized water (4 L) was spiked using a chlorpyrifos standard in acetone. Fortified water was diluted to give different final concentrations using deionized water. Spiked levels and percent recovery is given in Table 3.1. pH of distilled water was adjusted to 2 before the experiment as it is done for sample water.

Table 3-1: Recovery of chlorpyrifos from spiked water

Concentration of chlorpyrifos in water (ppb)	Chlorpyrifos in 200 mL water (ng)	Chlorpyrifos detected (ng)	Percent recovery
0.37	74	71.8	99.7
1.49	298	269.4	90.4
2.97	594	470.4	79.2
7.43	1486	1610.8	108.4

Average recovery is 94.4%

Instrument detection limits for water analysis for chlorpyrifos

Instrument noise for solvent = 1 mm (Figure 3.3)
Signal to Noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 12.5 ng/mL standard = 99 mm

Calculation

Minimum measurable peak height = 1 mm x 5
= 5 mm

Minimum measurable concentration based on 5 mm peak
= (12.5/99) x 5 ng/mL
= 0.631 ng/mL

Instrument detection limit = 0.7 ng/mL

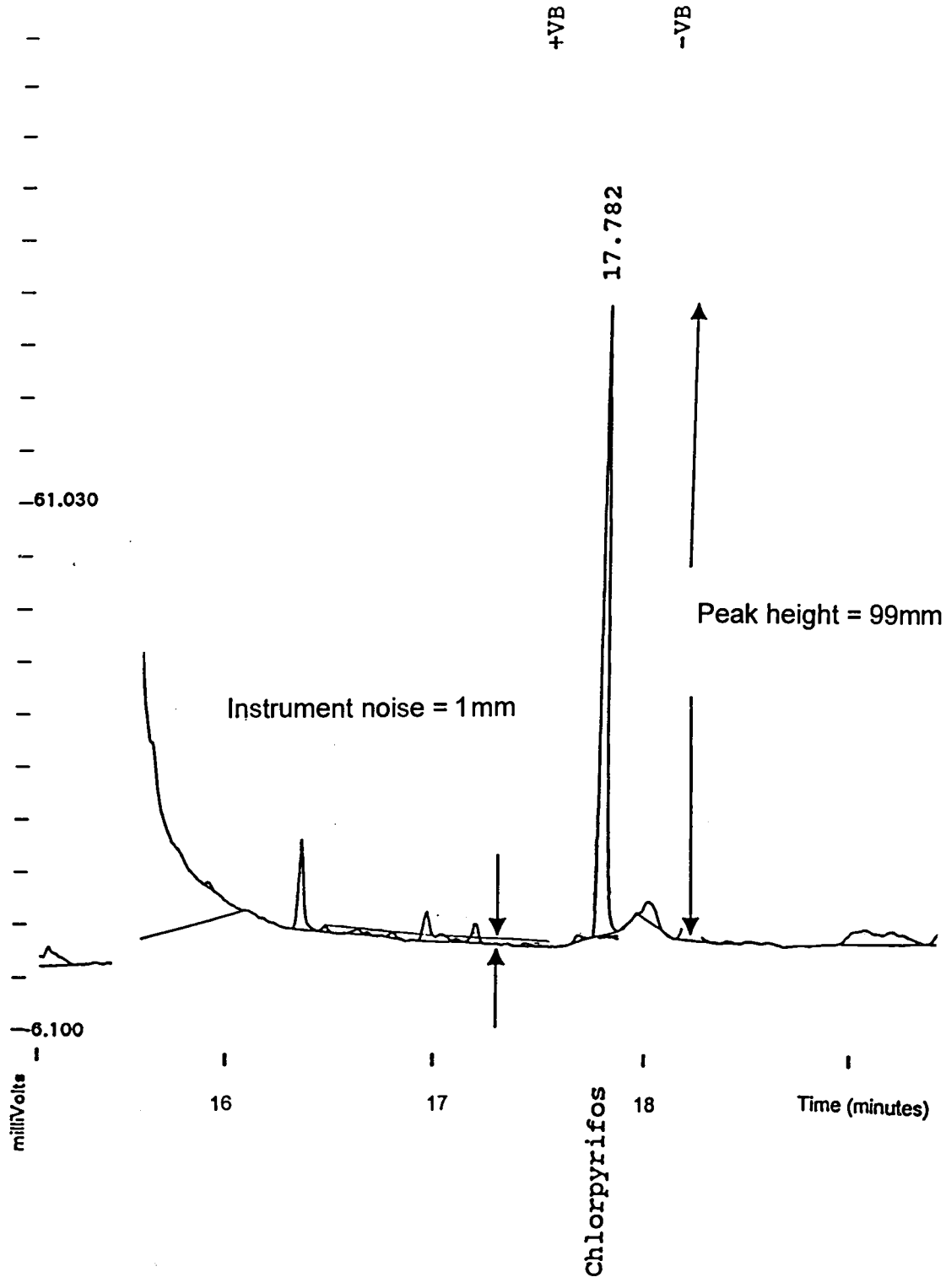

Figure 3.3: Chromatogram of 12.5ng/mL chlorpyrifos standard used to determine instrument detection limit (attenuation 50)

Method detection limits for water analysis for chlorpyrifos

Method noise = 2 mm (Figure 3.4)
Signal to Noise ratio = 5
Initial volume for the method = 200 mL
Final volume for the method = 1 mL
Peak height of the 12.5 ng/mL standard = 99 mm

Calculation

Minimum measurable peak height = 2 mm x 5
= 10 mm

Minimum measurable concentration based on 10 mm peak
= (12.5 ng/mL /99 mm) x10 mm
= 1.262 ng/mL

Since final volume is 1 mL , final concentration = 1.262 ng/mL

Initial volume is 200 mL, therefore,

Minimum amount of chlorpyrifos in 200 mL water = 1.262 ng

Minimum concentration in water = 1.262/200 ng/mL
= 6.13 ng/L in water

Method detection limit = 7 ng/L in water

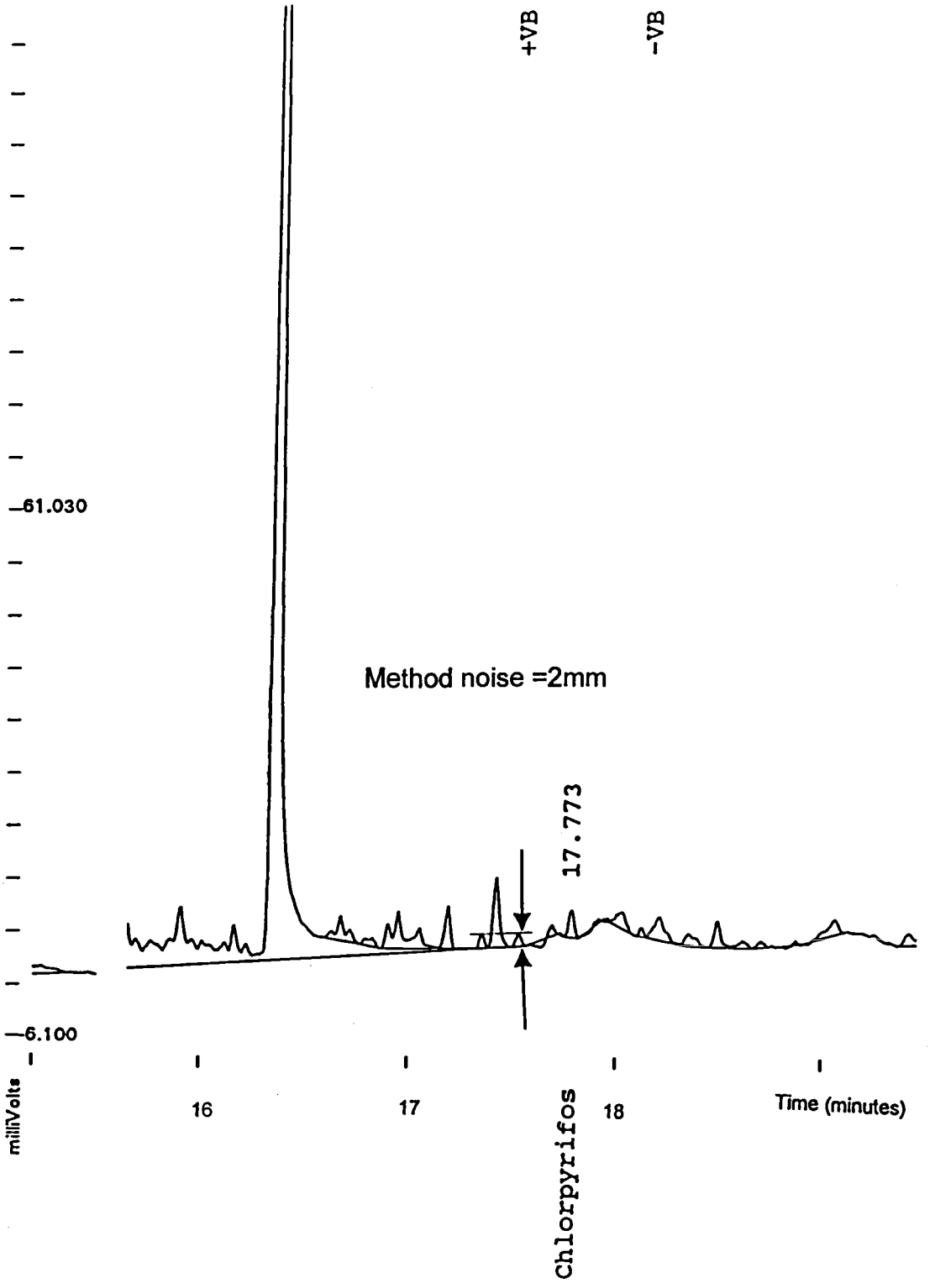

Figure 3.4: Chromatogram of blank water analysis used to determine method detection limit (attenuation 50)

HOUSE DUST ANALYSIS FOR CHLORPYRIFOS

The method used was a modified version of Sydney et al. (1999) and EPA method 525.2. Cotton balls were extracted with 2 x 5 mL aliquots of acetone and the extracts combined. Cotton balls were placed in tubes with caps (diameter of ~1 cm), 5 mL of acetone was added (acetone level is above cotton) and sonicated for 30 min (Figure 3.5-A). Sonicated tubes were inverted into larger tubes (diameter of ~2 cm) and centrifuged for 5 min at 10,000rpm (Figure 3.5-B) allowing only acetone to drain into the large tube. Cotton was pressed down in the small tube using a spatula to avoid moving down during centrifuge. A small glass stopper was placed on the bottom of the large tube to make enough space for acetone to drain. Acetone extracts were transferred to volumetric tubes using disposable pipettes (Figure 3.5-C and D). Small tubes were re-centrifuged if necessary to recover at least 4 mL from each extract. About 9 mL of acetone was recovered from cotton from both extracts. The acetone extracts (8 mL) were diluted with 200 mL of water (pH adjusted to 2 using 6N H_2SO_4) keeping the same ratio (of 4 mL of acetone diluted in 100 mL of water) described by Sydney et al. (1999). 1 mL (5% of total volume of water) of methanol was also added to each acetone water mixture.

Sample cleanup was performed using 1g of octadecyl (^{18}C) in solid phase extraction (SPE) columns (manufactured by Baker Bond). SPE columns were conditioned by eluting each cartridge with a 5 mL aliquot of ethyl acetate followed by a 5 mL aliquot of methylene chloride. The cartridge was allowed to drain dry after each flush. Then each cartridge was eluted with a 10 mL aliquot of methanol, not allowing the methanol to elute below the top of the cartridge packing. Ten mL aliquots of de-ionized water were added to the cartridge, but before the water level droped below the top edge of the packing, sample water was added to the reservoir (EPA 525.2). Columns were dried under vacuum for 40 min (Sydney et al., 1999) to make sure no more water

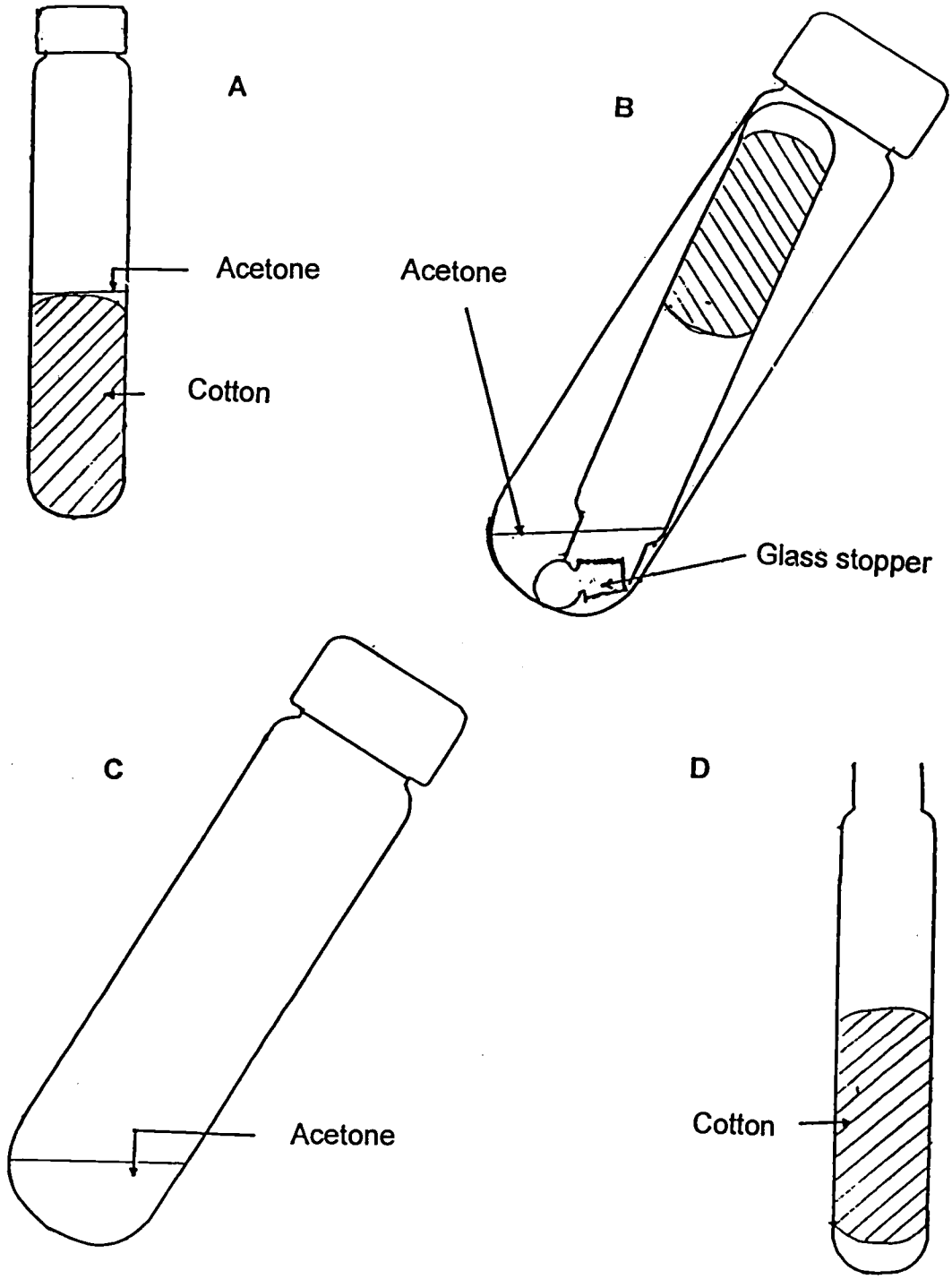

Figure 3.5: Extraction of cotton with acetone

was present. The elution apparatus (15 mL glass tube) was set under the column to collect eluants. Five mL aliquots of ethyl acetate was transferred after making sure container was free of water and rinsed inside. The solvent was used to elute columns. The same steps were followed with a 5 mL aliquot of methylene chloride. As a final rinse, 2 mL of methylene chloride was passed through the columns. Eluants were collected, combined and concentrated to 1 mL before analysis.

Fortified dust in cotton: Cotton balls were used to collect dust from the front porch of none agricultural area for the recovery studies. Four levels of chlorpyrifos-spiked dust (in cotton) were extracted with samples to validate the method. Spike levels and percent recovery are listed in Table 3.2.

Table 3.2: Recovery of chlorpyrifos from spiked cotton balls (with and without dust)

Chlorpyrifos in cotton ball (ng)	Chlorpyrifos in 200 mL water (ng)	Chlorpyrifos recovered (ng)	Percent recovery
250.0	181.8	134.5	74
100.0	72.7	51.6	71
25.0	18.2	12.9	71
0	0	0	-

Average recovery 72%

* only cotton (no dust)

Instrument detection limits for dust analysis for chlorpyrifos

Instrument noise for solvent = 2 mm (Figure 3.6)
Signal to noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 25 ng/mL standard = 104 mm

Calculation

Minimum measurable peak height = 2 mm x 5
= 10 mm

Minimum measurable concentration based on 10 mm peak
= (25 ng/mL/ 104 mm) x 10 mm

Instrument detection limit = 3 ng/mL

Method detection limits for dust analysis for chlorpyrifos

Method noise = 10 mm (Figure 3.7)
Signal to Noise ratio = 5
Final volume for the method = 1 mL
Peak height of the 25 ng/mL standard = 104 mm

Calculation

Minimum measurable peak height = 10 mm x 5
= 50 mm

Minimum measurable concentration based on 50 mm peak
= (25 ng/mL /104 mm) x 50 mm

Since final volume is 1 mL, final concentration = 12.019 ng/mL

Initial floor area is $2ft^2$, minimum amount in 2 ft^2 area is = 12.019 ng

Method detection limit = 2 ng/ft^2

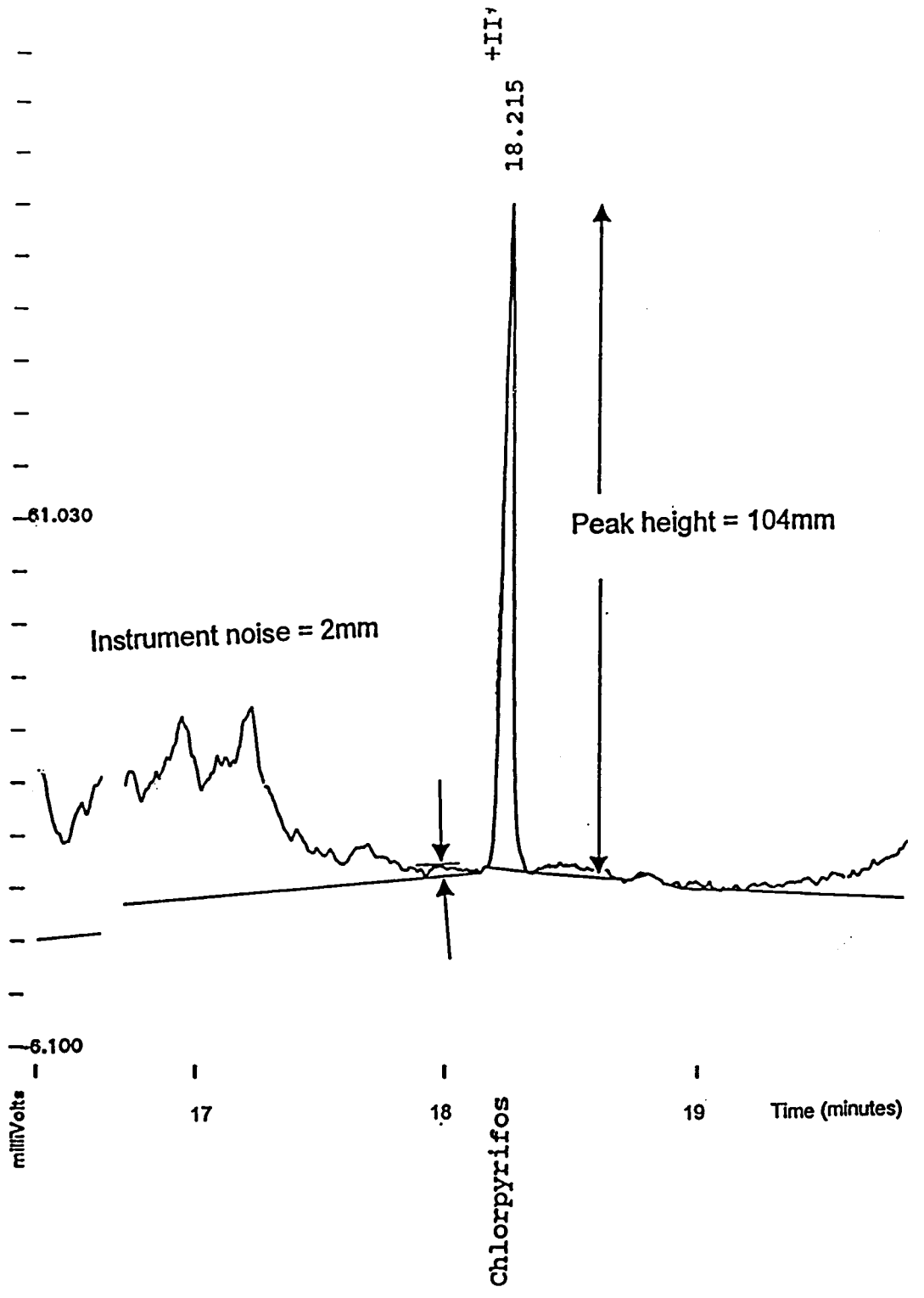

Figure 3.6: Chromatogram of 25 ng/mL chlorpyrifos standard used to determine instrument detection limit in dust analysis for chlorpyrifos (attenuation 50)

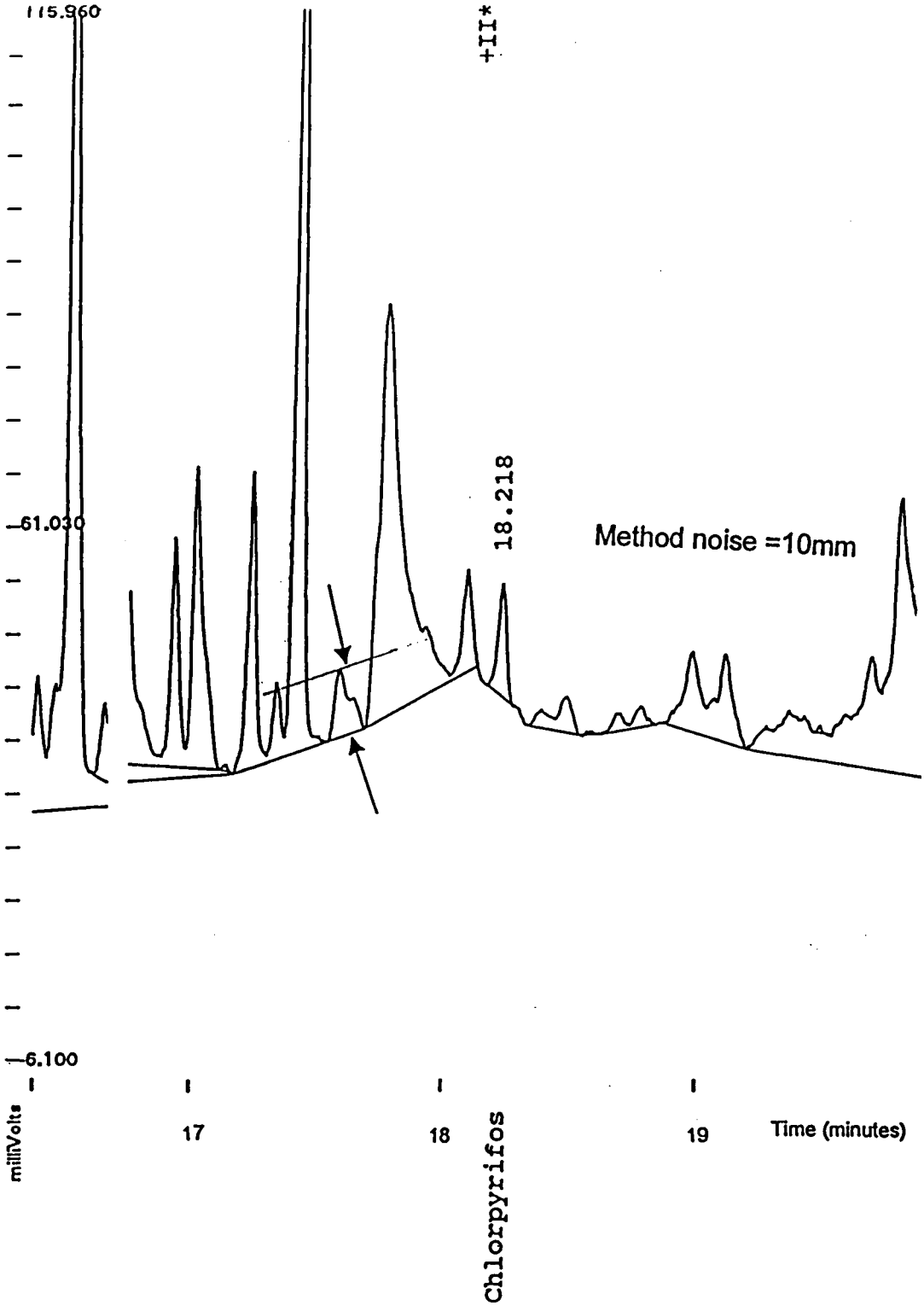

Figure 3.7: Chromatogram of blank dust extract used to determine method detection limit in dust analysis for chlorpyrifos (attenuation 50)

Gas chromatography conditions: A varian workstation 3600 gas chromatographic system with two electron capture detectors was used for the analysis of chlorpyrifos in water and dust. Two columns DB1 (non-polar) and DB-XLB (polar) were attached to the same injection port, and the responses on both columns were compared to confirm the presence of chlorpyrifos. Both columns were 30 m in length, 0.25 mm (internal diameter), and 0.25μm film thickness manufactured by J&W scientific, USA. Ultra-pure helium and 99.9% pure nitrogen gas were used as carrier and makeup, respectively. Two micro liter samples were injected using a split-less injection system of the Varian auto sampler 8200. Temperature at injector port, and detector were 50 and 350 ^{0}C, respectively. The 3-step column temperature program was 100, 190, and 250^{0} C for 6, 2.5, and 3 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 250 at 20 ^{0}C/min.

Standard curve: Standard curve (Figure 3.8) was generated using six different concentrations and repeated with each sample set.

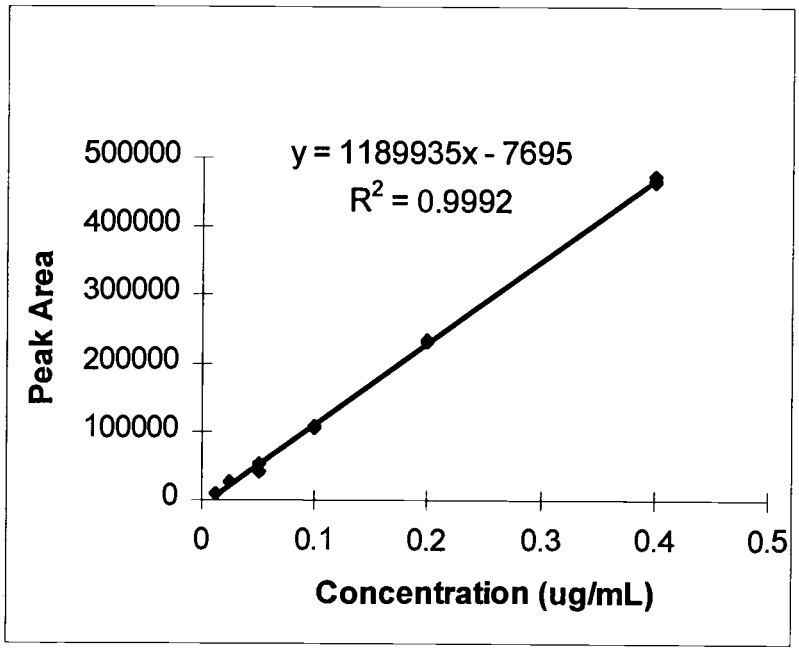

Figure 3.8: A standard curve generated for chlorpyrifos

ANALYSIS OF TCP FROM WATER

Water (15 mL) was transferred to glass tubes (25 mL) and acidified with two drops of 6 M sulfuric acid. Then, 0.4 g of sodium chloride was added to each tube before extracting twice with 5 mL of benzene. Benzene layers were removed using disposable pipettes, and the extracts combined, and concentrated to 1 mL. 5 μL of N,O-bis(trimethylsilyl)acetamide (BSA) was added just before injecting 2 μL in to the varian gas chromatograph system.

Recovery study: A volume of 4 L of water was fortified with a TCP standard in acetone, and different dilutions with deionized water were used for recovery studies. Final concentrations of TCP in spiked water and percent recoveries are given in Table 3.3.

Table 3.3: Recovery of TCP from spiked water

Concentration of TCP in water (ppb)	TCP in 15mL water (ng)	TCP recovered (ng)	Percent recovery
16.4	246.1	184.3	75
9.8	147.7	131.6	89
6.6	98.4	93.6	95
3.3	49.2	55.8	88

Average recovery 86.8%

Instrument detection limits for water analysis for TCP

Instrument noise for solvent = 3 mm (Figure 3.9)
Signal to noise ratio = 5

Final volume for the method = 1 mL
Peak height of the 10 ng/mL standard = 100 mm

Calculation

Minimum measurable peak height = 3 mm x 5
= 15 mm

Minimum measurable concentration based on 15 mm peak
= (10 ng/mL/100 mm) x 15 mm
= 1.5 ng/mL

Instrument detection limit = 2 ng/mL

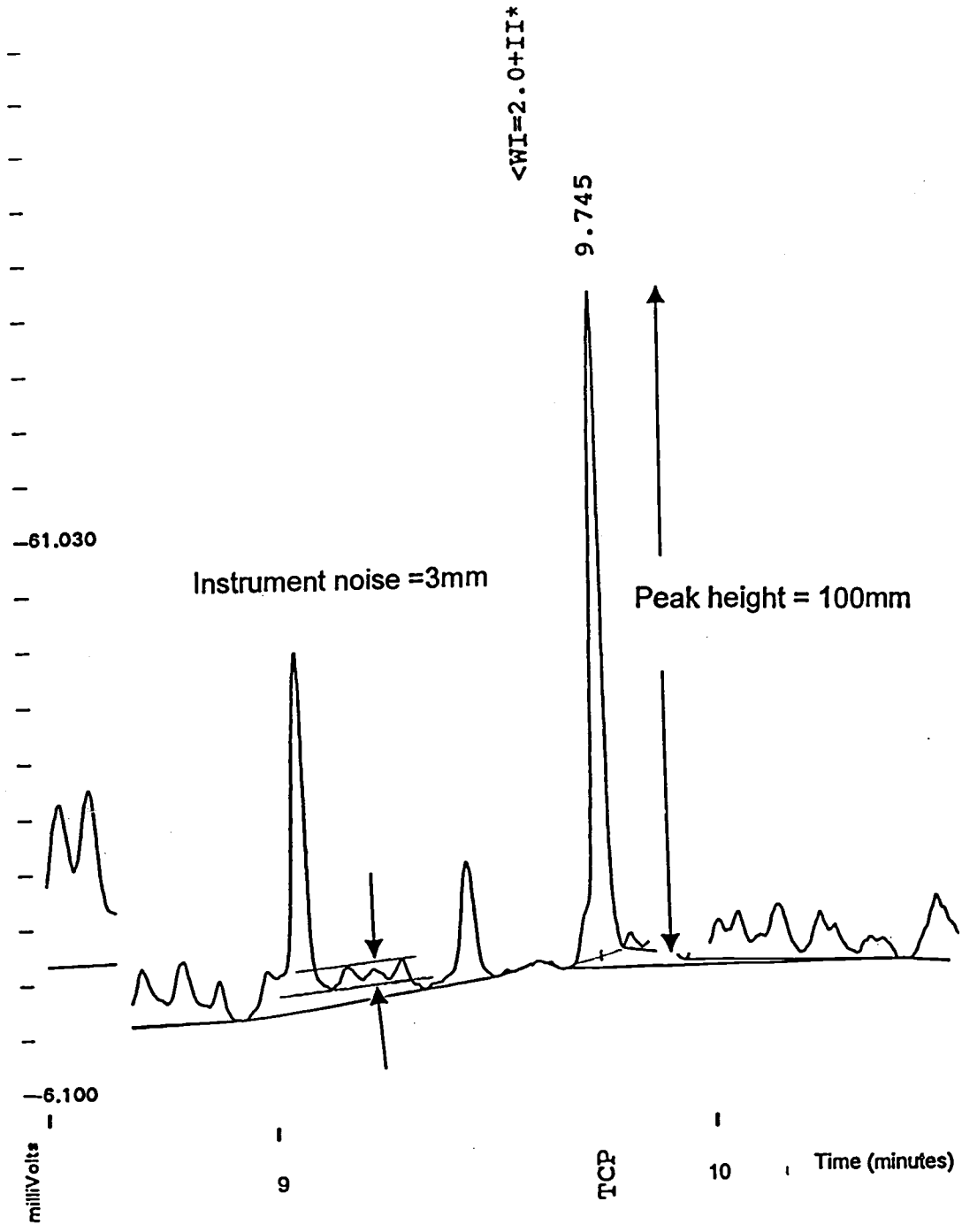

Figure 3.9: Chromatogram of 10ng/mL TCP standard used to determine instrument detection limit in water analysis for TCP (attenuation 50)

Method detection limits for water analysis for TCP

Method noise	= 5 mm (Figure 3.10)
Signal to noise ration	= 5
Initial volume for the method	= 15 mL
Final volume for the method	= 1 mL
Peak height of the 10 ng/mL standard	= 100 mm

Calculation

Minimum measurable peak height = 5 mm x 5

= 25 mm

Minimum measurable concentration based on 25 mm peak

= (10 ng/mL /100 mm) x 25 mm

= 2.5 ng/mL

Since final volume is 1 mL , final concentration = 2.5 ng/mL

Initial volume is 15 mL; therefore,

Minimum amount of TCP in 15 mL water = 2.5 ng

Minimum concentration in water = 2.5/15 ng/mL

Method detection limit = 167 ng/L in water

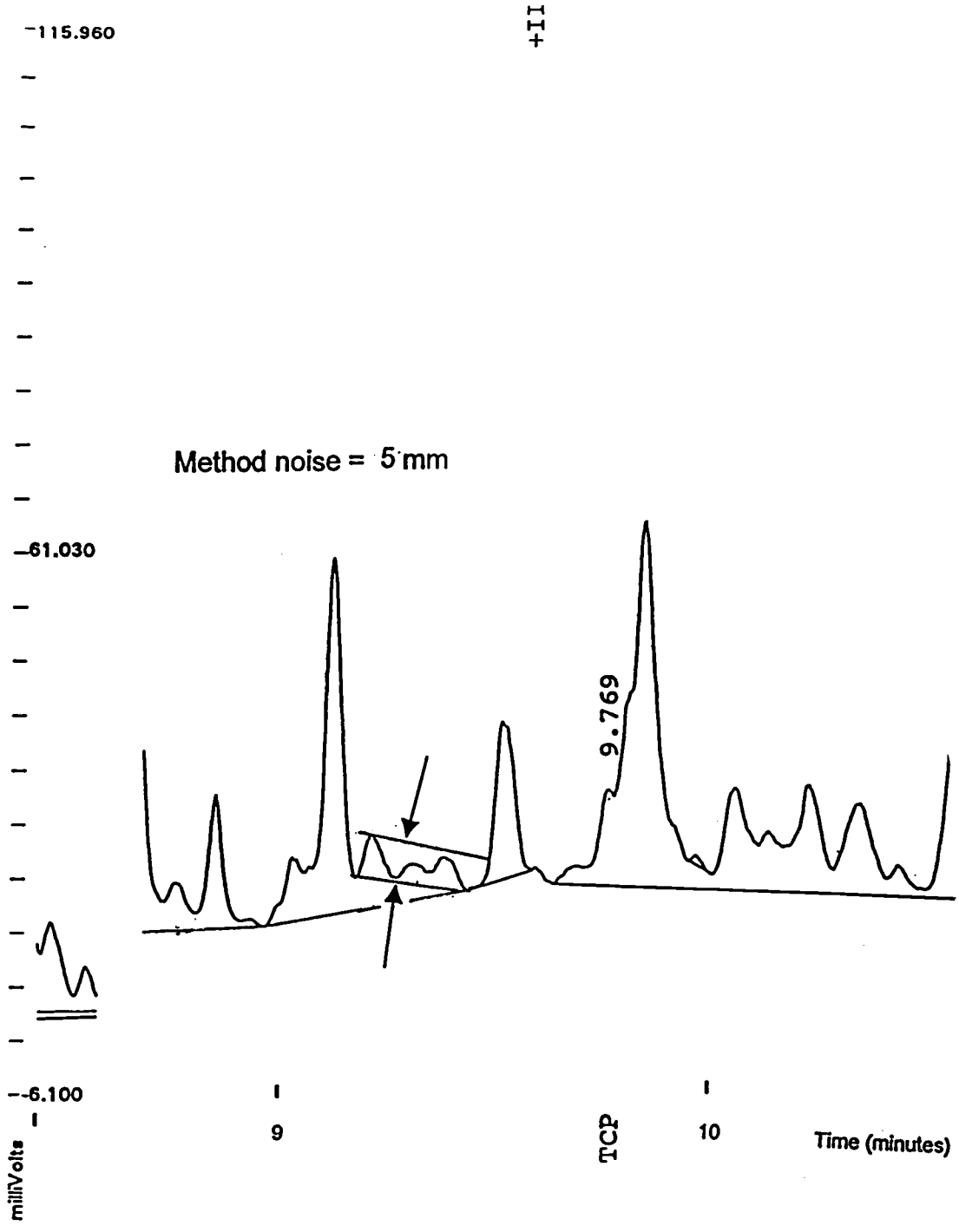

Figure 3.10: Chromatogram of blank water extract used to determine method detection limit in water analysis for TCP (attenuation 50)

Gas chromatography analysis: The same Varian work station 3600 gas chromatographic system was used. Temperature at injector port and detector were 50 and 350 ^{0}C, respectively. The 3-step column temperature program was 100, 190, and 300 ^{0}C for 6, 2.5, and 2 min, respectively. The rates of temperature increases were 100 to 190 at 20 ^{0}C/min and 190 to 300 at 25 ^{0}C/min.

Standard curve: Standard curve was generated using four points and the curve was reproduced with each set of samples. A standard curve used for calculations is given in Figure 3. 11.

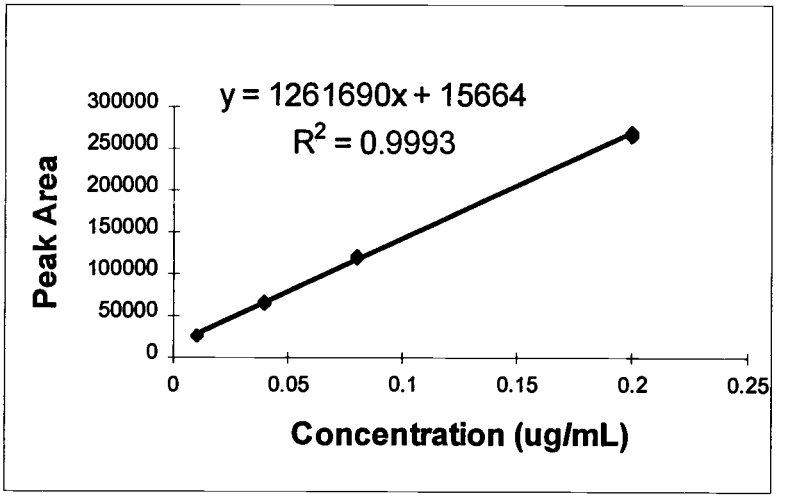

Figure 3.11: Standard curve for TCP

RESULTS

Drinking water analysis for chlorpyrifos: The method detection limit for chlorpyrifos in water was 7 ng/L and the mean recovery of chlorpyrifos from spiked water was 94.4% (Table 3.1). Retention time for chlorpyrifos on DB-1 column was 17.782 min for the method. Spiked water samples were analyzed along with drinking water samples. Chromatograms from the DB-1 column for sample water, blank water analysis, and chlorpyrifos standard are given in Figure 3.12(a-e). None of the drinking water samples contained quantifiable amounts of chlorpyrifos.

Figure 3.12: Chromatograms for drinking water analysis for chlorpyrifos

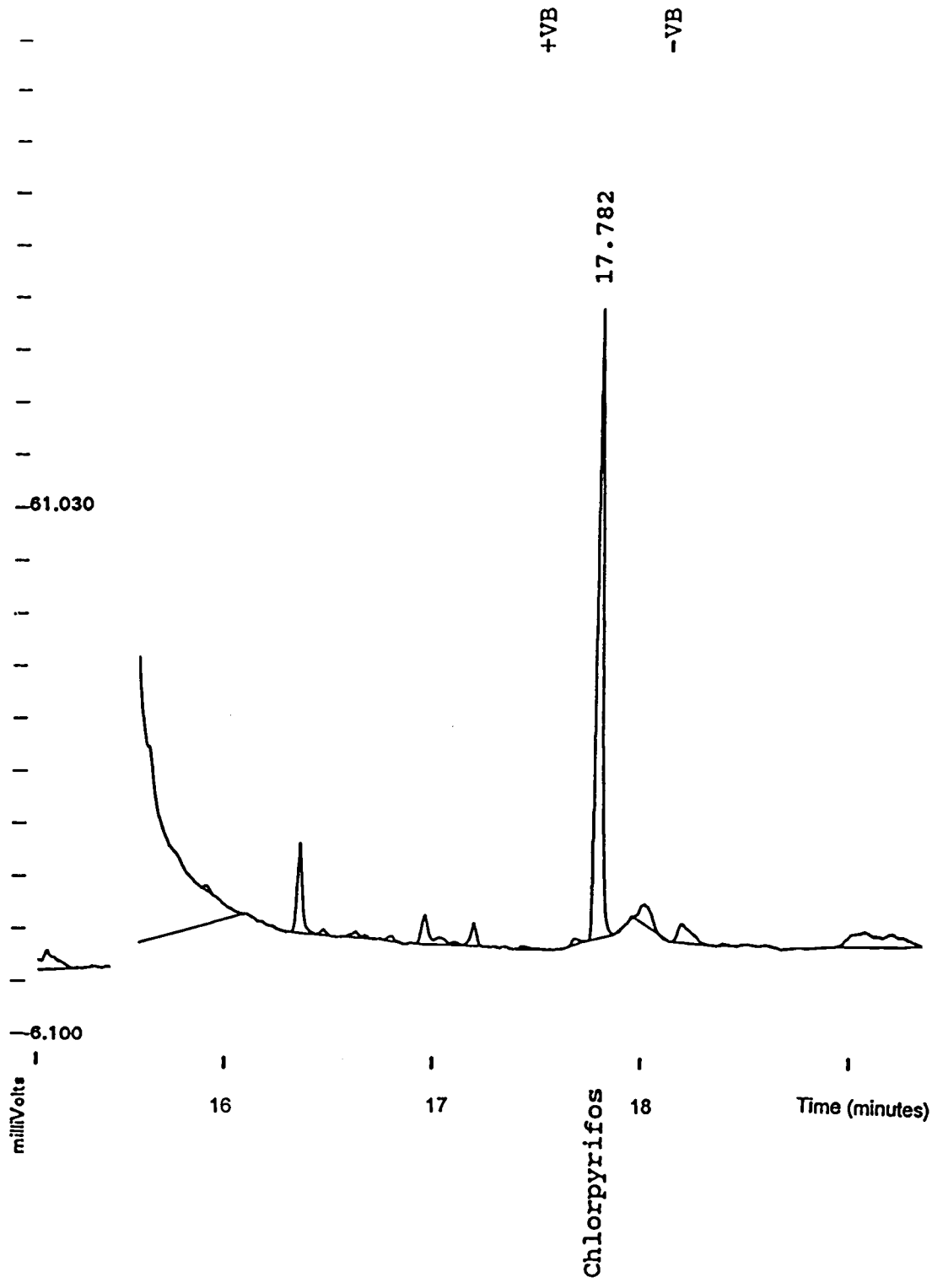

Figure 3.12a: Chromatogram of 12.5ng/mL chlorpyrifos standard (attenuation 50)

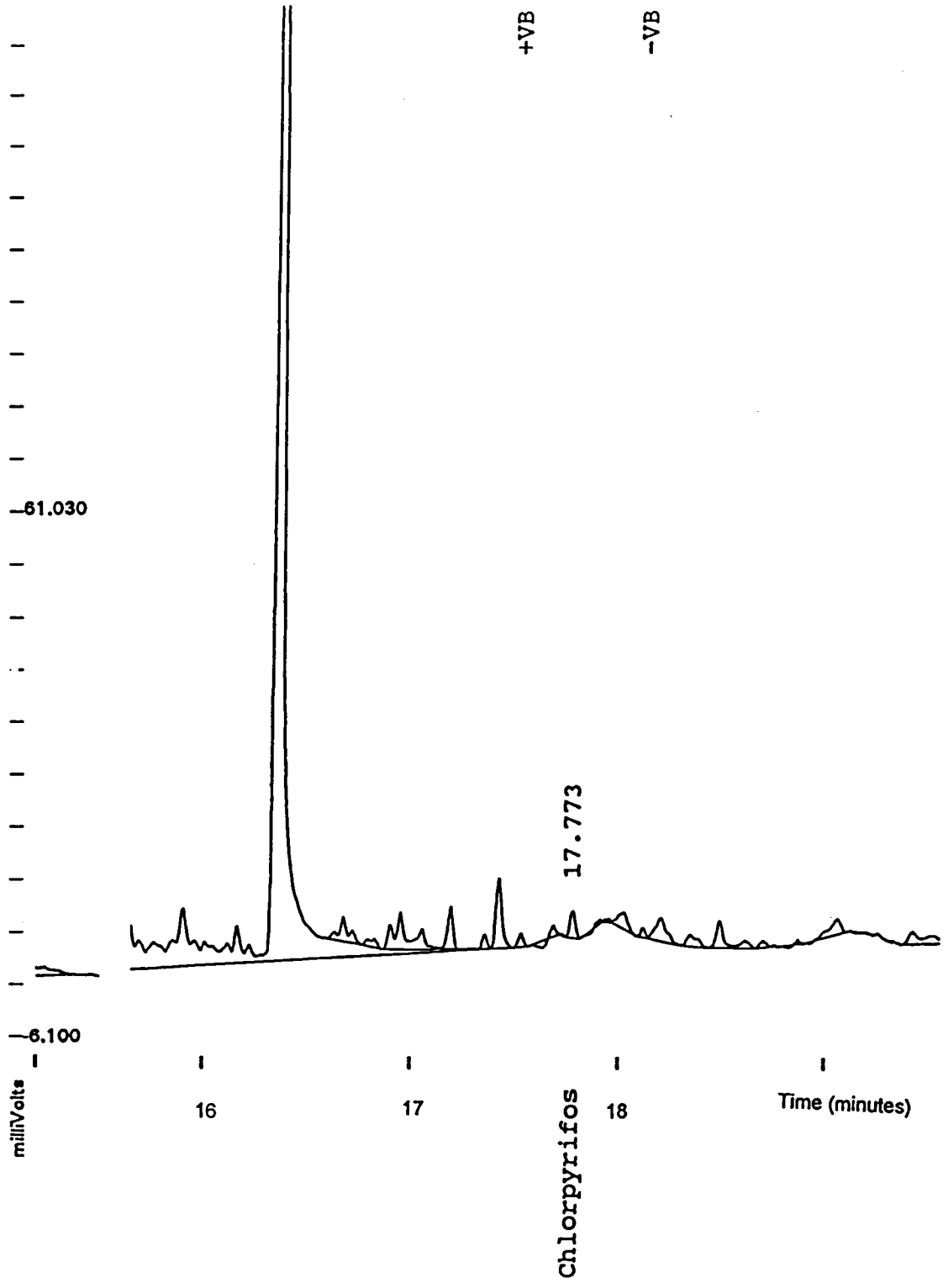

Figure 3.12b: Chromatogram of blank water analysis for background level of chlorpyrifos (attenuation 50)

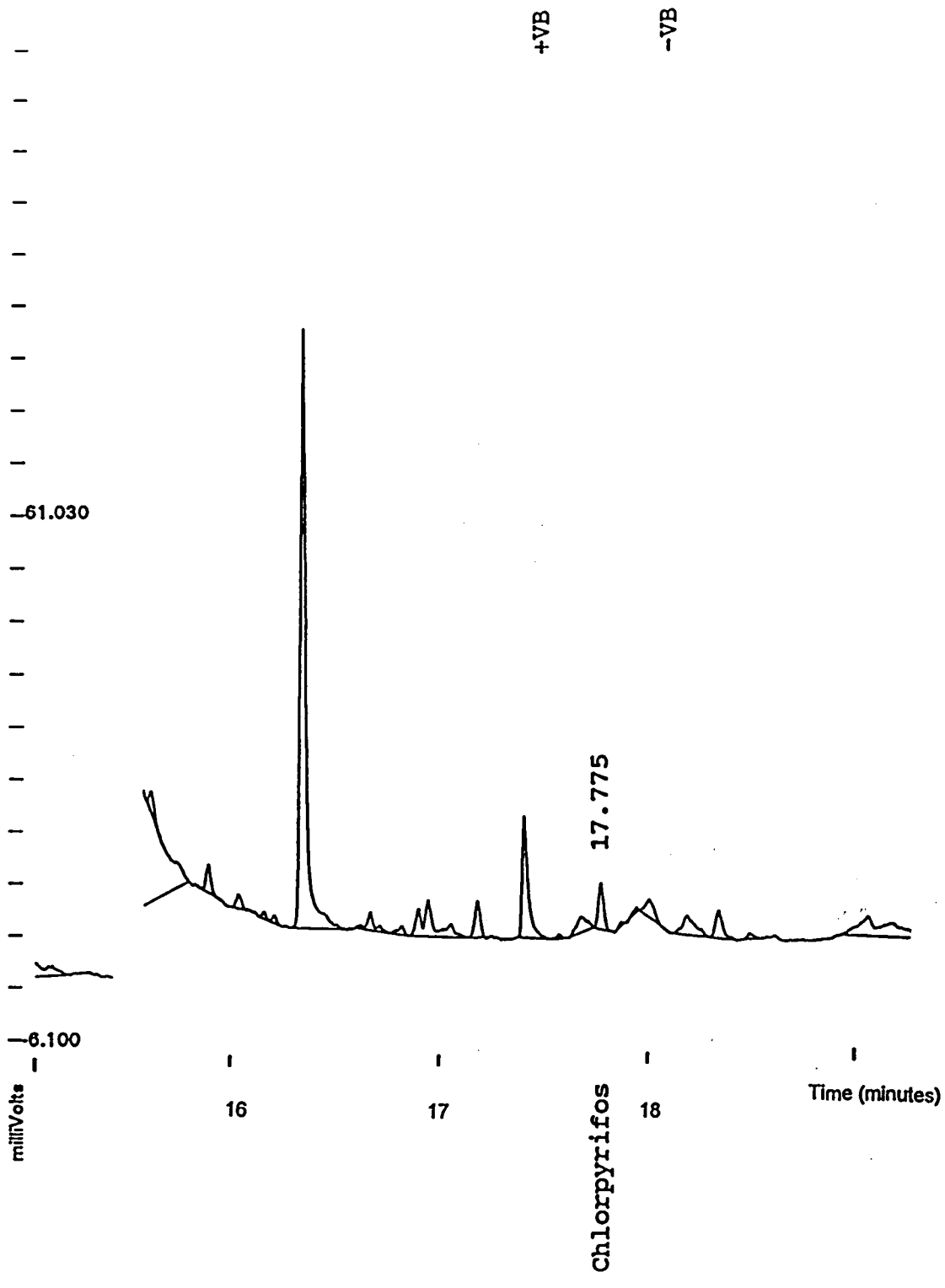

Figure 3.12c: Chromatogram of water (from well 1) analysis for chlorpyrifos (attenuation 50)

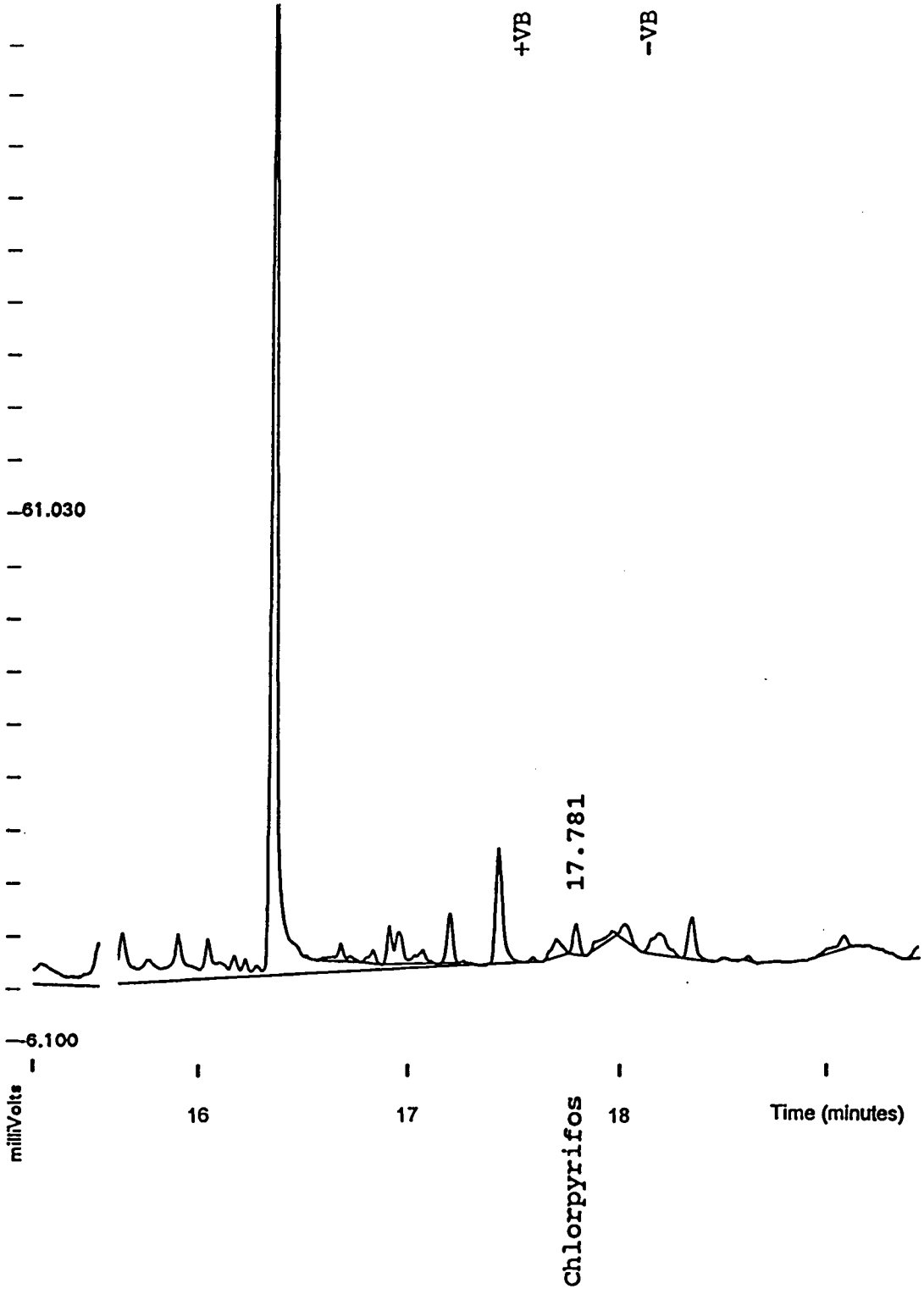

Figure 3.12d: Chromatogram of water (from well 2) analysis for chlorpyrifos (attenuation 50)

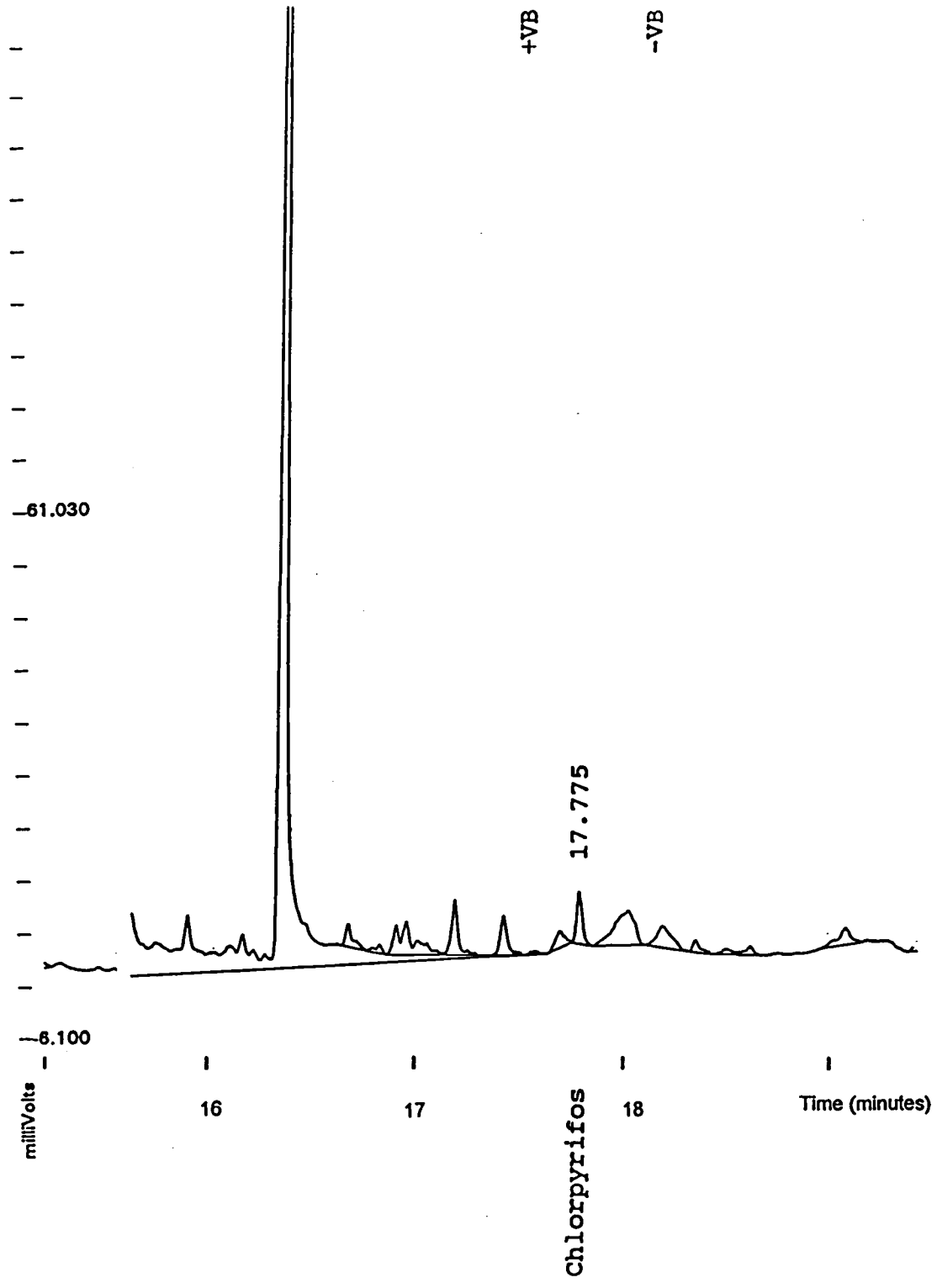

Figure 3.12e: Chromatogram of water (from well 3) analysis for chlorpyrifos (attenuation 50)

Dust analysis for chlorpyrifos:. In the dust analysis, method detection limit was 2 ng/ft^2, and the mean recovery from spiked cotton was 72%. Retention time for chlorpyrifos was 18.215 min on a DB-1 column, and 15.227 min for a DB-XLB column. The electron capture detector (ECD) is a very sensitive but has limited specificity. This detector responds to any electrophilic compound and peaks were found in all dust samples. Dust samples gave peaks with retention time of chlorpyrifos on a DB-XLB column in samples from all locations of each house. The same samples (same injection) did not show comparable peaks on the DB-1 column with respect to magnitude and retention time. In Figure 3.13b (DB-XLB column), the peak at 15.227 min (dust for front porch) is lower than the 0.05 μg/mL standard. From the same injection on the DB-1 column (Figure 3.13a) the peak is higher than the 0.05 μg/mL standard and shows a retention time different from chlorpyrifos. When Figures 3.15a and 13.15b are considered, front porch samples do not have the same retention time as chlorpyrifos in DB-1 column, but on DB-XLB the same sample had a peak similar to chlorpyrifos. The magnitudes of those two peaks were also different. Double peaks were found (one peak having retention time close to chlorpyrifos) on DB-1 column for dust sample from living room (Figure 3.13a). The same sample gave a peak identical with chlorpyrifos standard in DB-XLB (Figure 3.13b) suggesting the possibility of a small amount of chlorpyrifos in that sample. Similarly, in Figure 3.15a a sample from the kitchen (DB-1 column) had a double peak (a little peak with retention time close to chlorpyrifos), but the same injection gave a larger peak identical to chlorpyrifos in BD-XLB column (Figure 3.15b). There was no consistent response among sampling sites in the same house. A comparison among houses indicated that samples from kitchens (K1,K2 and K3) had suspect peaks in both columns. Analytical results do not provide any convincing evidence for the presence of chlorpyrifos in house dust. It was not possible to use more selective but less sensitive detector to establish whether the response was due to the phosphorus containing compounds.

Figure 3.13: Comparison of dust analysis results from house 1

1F - Dust from front porch
1L - Dust from living room
1K - Dust from kitchen

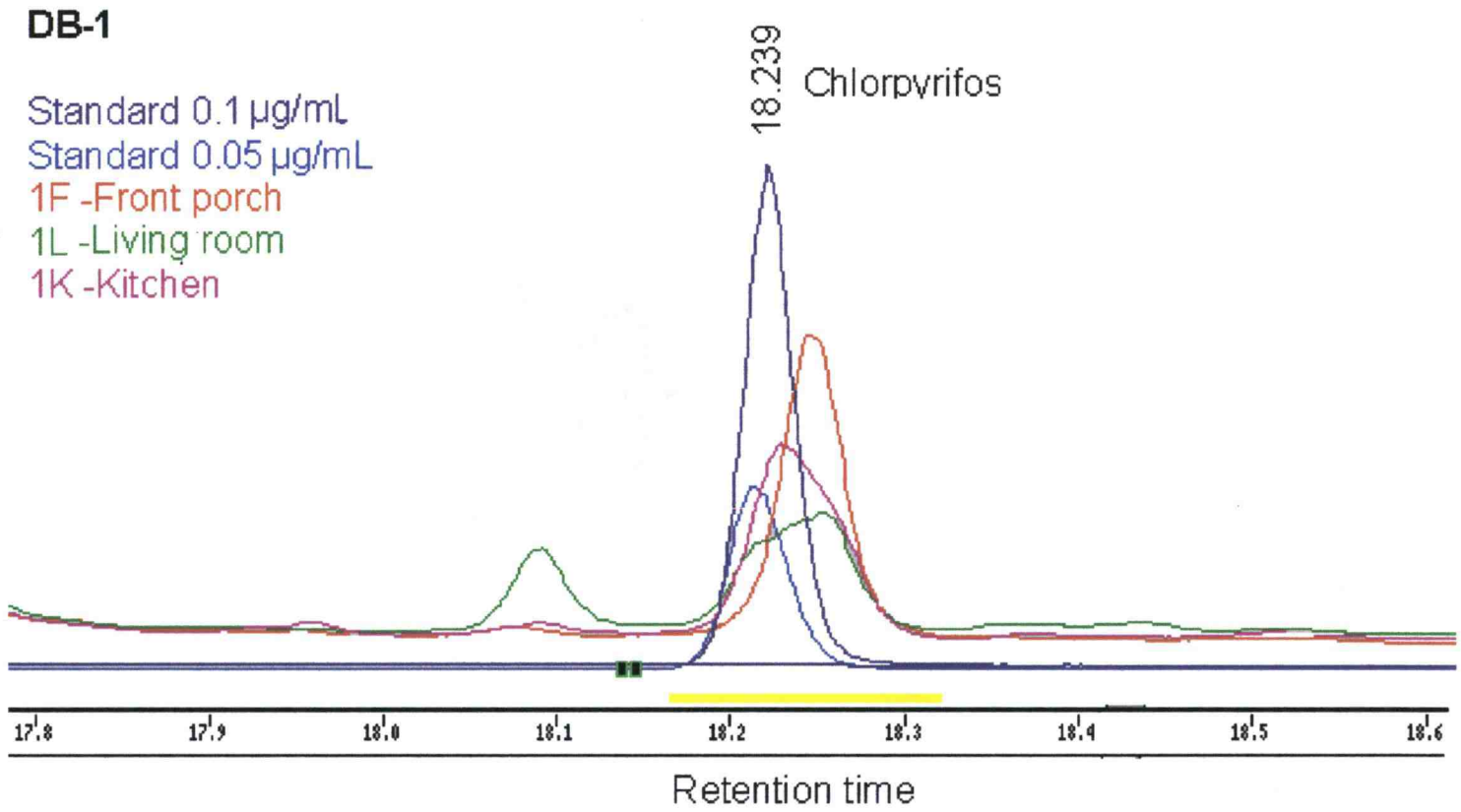

Figure 3.13a: Comparison of dust analysis results from house 1 on DB-1 column

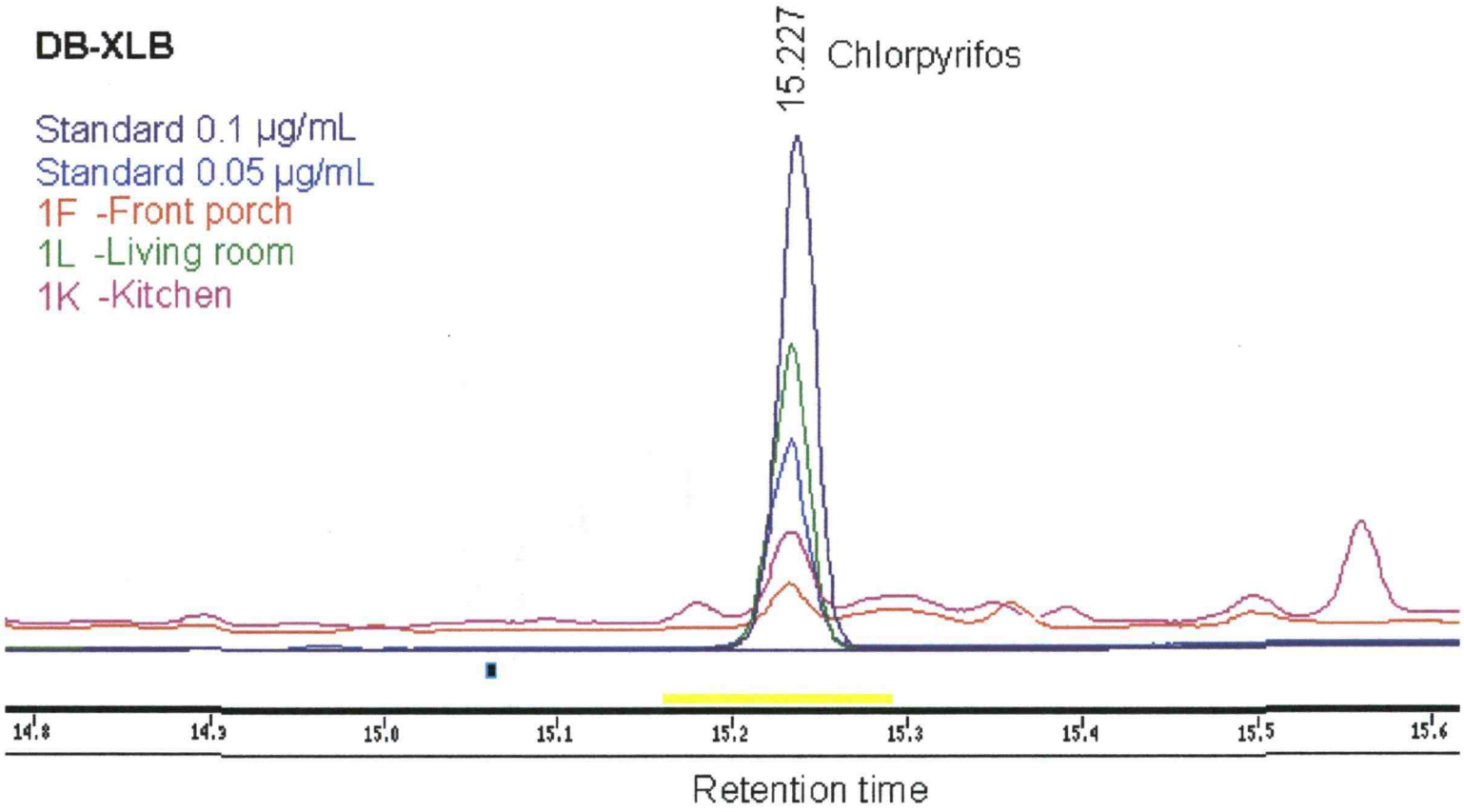

Figure 3.13b: Comparison of dust analysis results from house 1 on DB-XLB column

Figure 3.14: Comparison of dust analysis results from house 2

2F - Dust from front porch
2L - Dust from living room
2K - Dust from kitchen

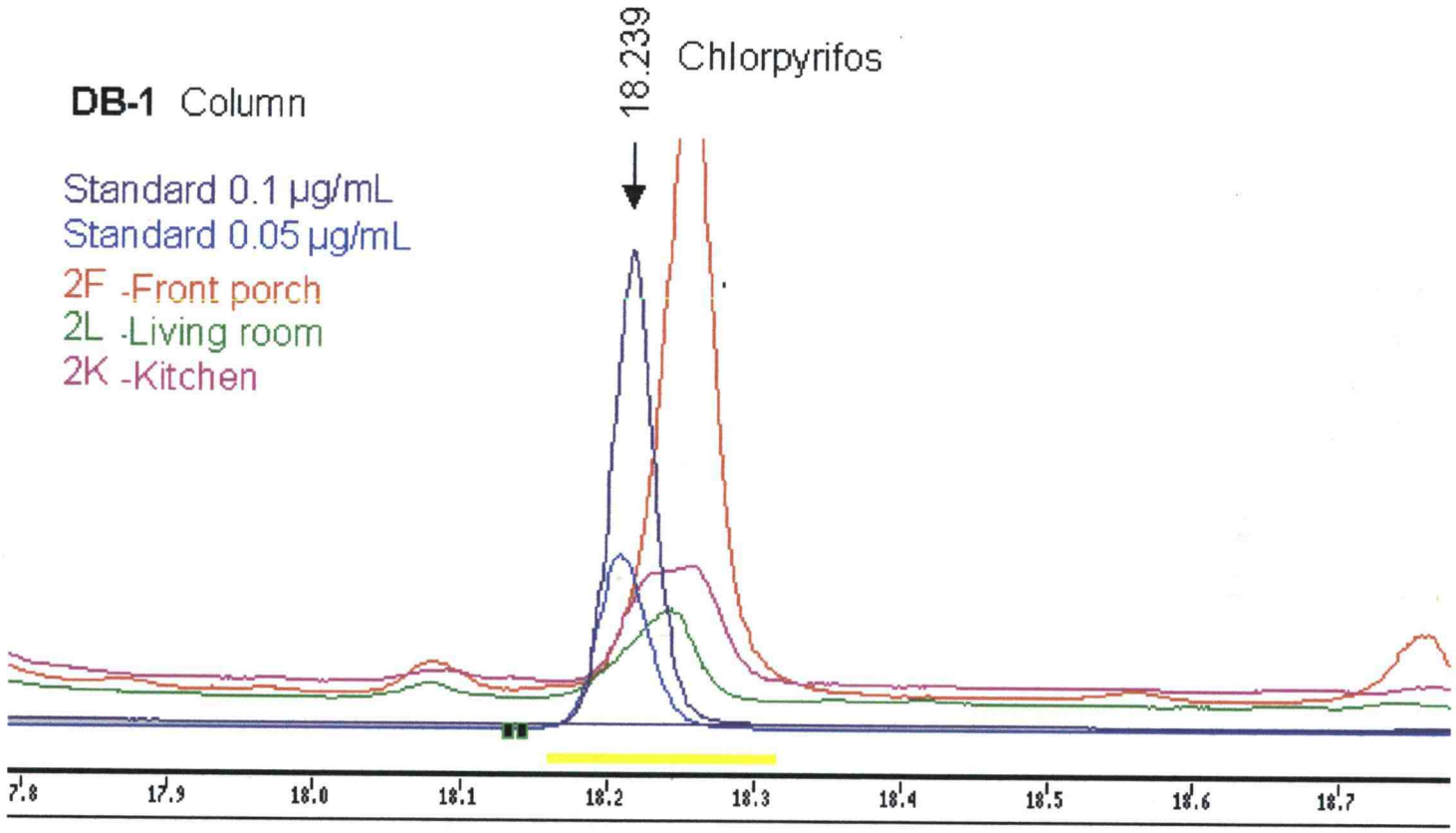

Figure 3.14a: Comparison of dust analysis results from house 2 on DB-1 column

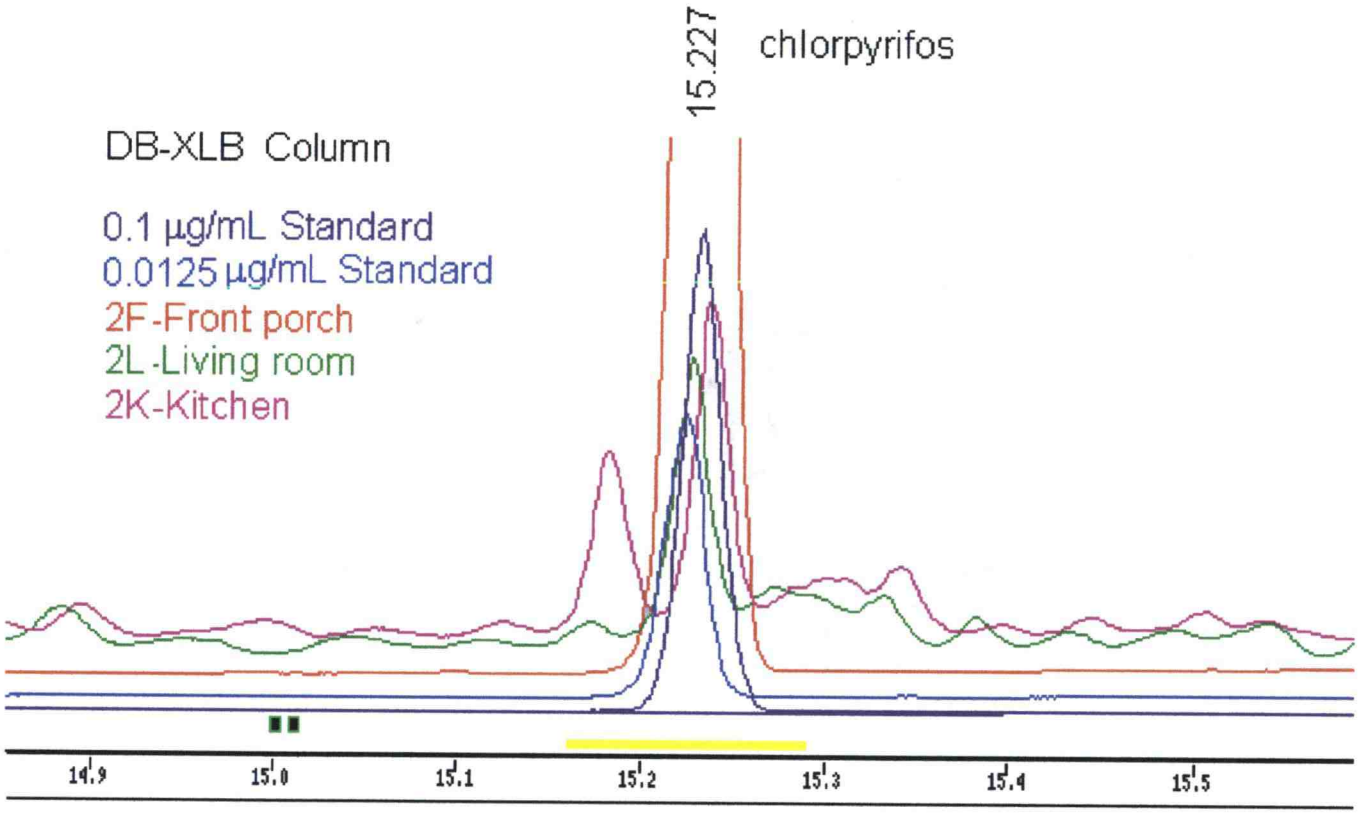

Figure 3.14b: Comparison of dust analysis results from house 2 on DB-XLB column

Figure 3.15: Comparison of dust analysis results from house 3

3F - Dust from front porch
3L - Dust from living room
3K - Dust from kitchen

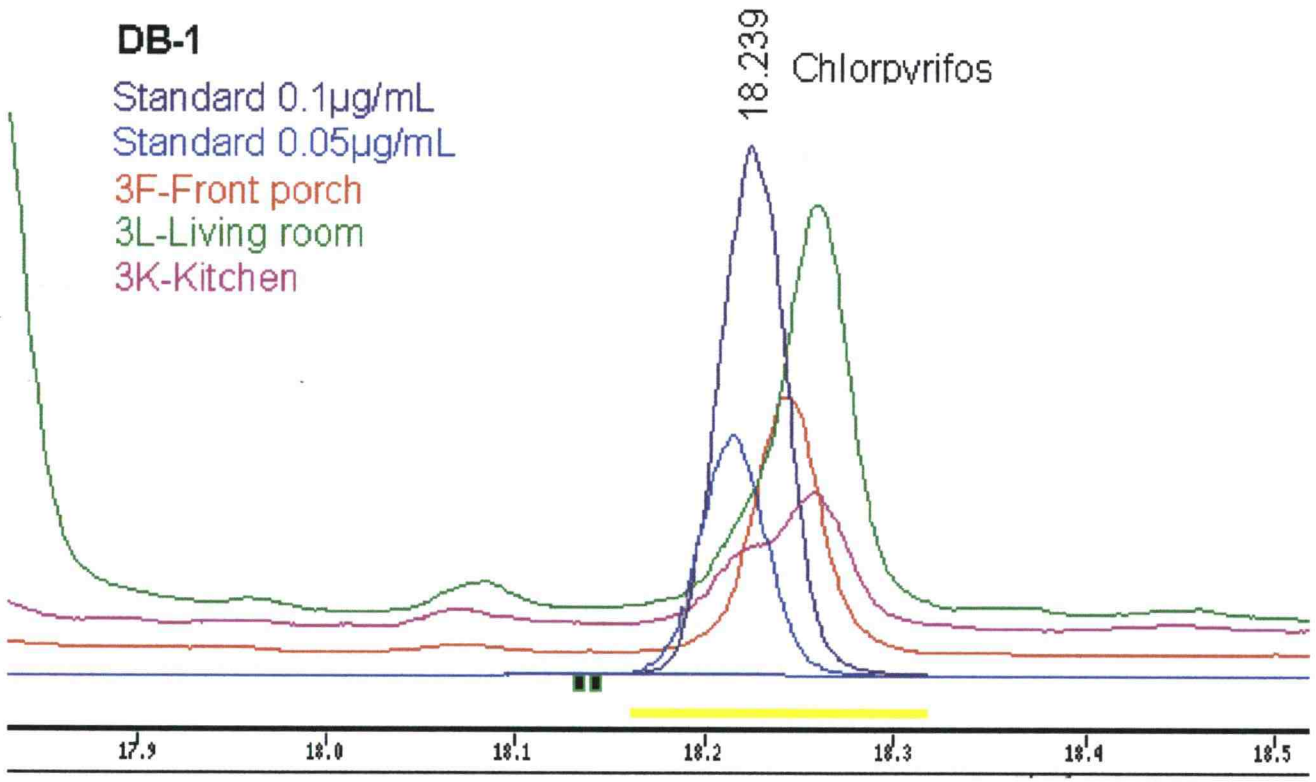

Figure 3.15a: Comparison of dust analysis results from house 3 on DB-1 column

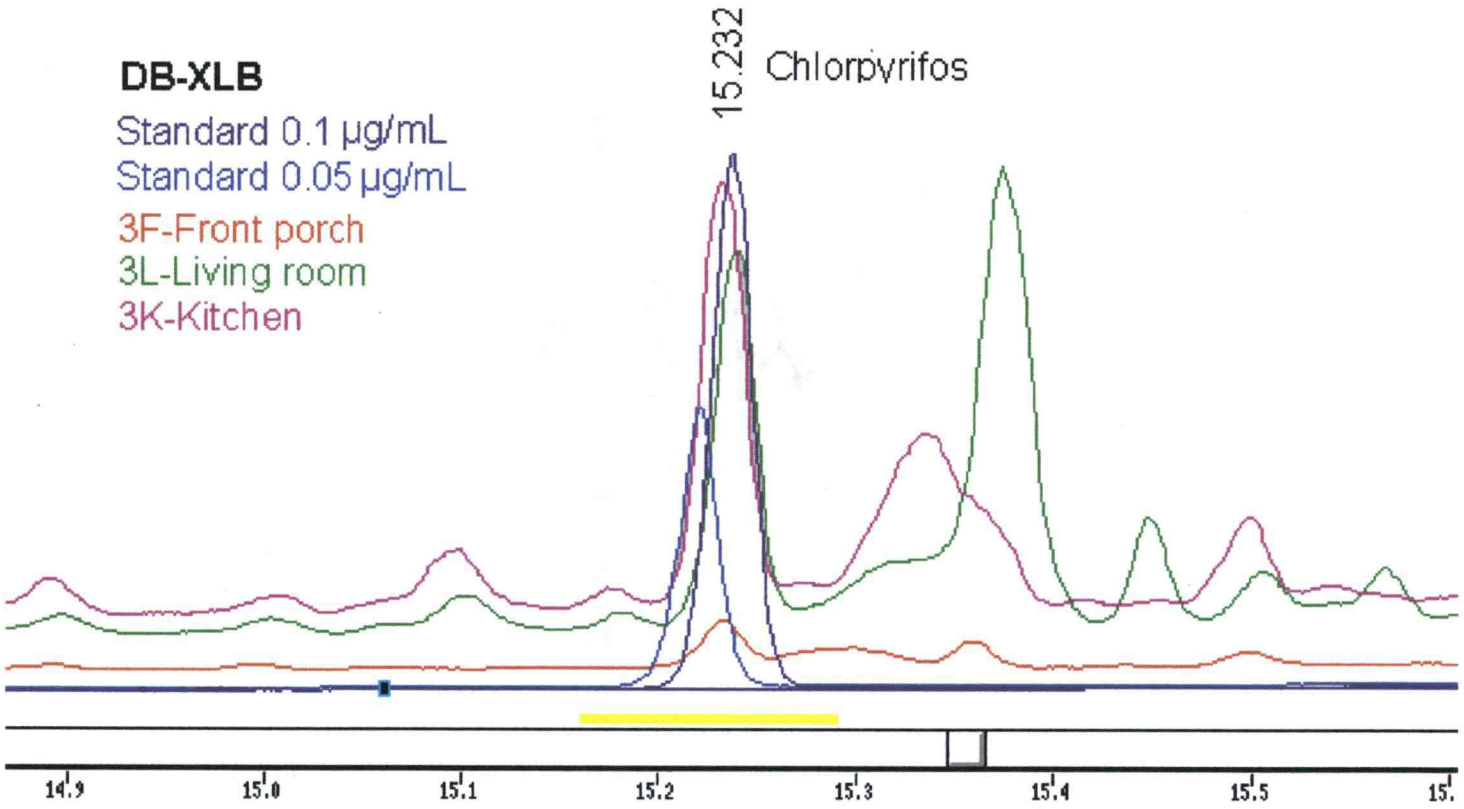

Figure 3.15b: Comparison of dust analysis results from house 3 on DB-XLB column

Water analysis for TCP: The Method detection limits for TCP in water was 167 ng/L and the mean recovery from spiked water samples was 86.5%. Retention time for TCP was 12.499 min on the DB-1 column. Two out of three wells were located at the center of the farm field, and the levels of TCP in those two were 9 and 10 ng/mL, respectively. The third well was located about 300m away from the cultivated area, and the level of TCP was 0.6 ng/mL. TCP in water was either from TCP leaching from soil into the well or hydrolysis of chlorpyrifos in the drinking water wells. Chromatographs for TCP analysis in drinking water samples, TCP spiked blank water (49 ng TCP in 15 mL water), and TCP standard are given in Figure 3.16 (a-e).

Figure 3.16: Chromatograms for water analysis for TCP in drinking water

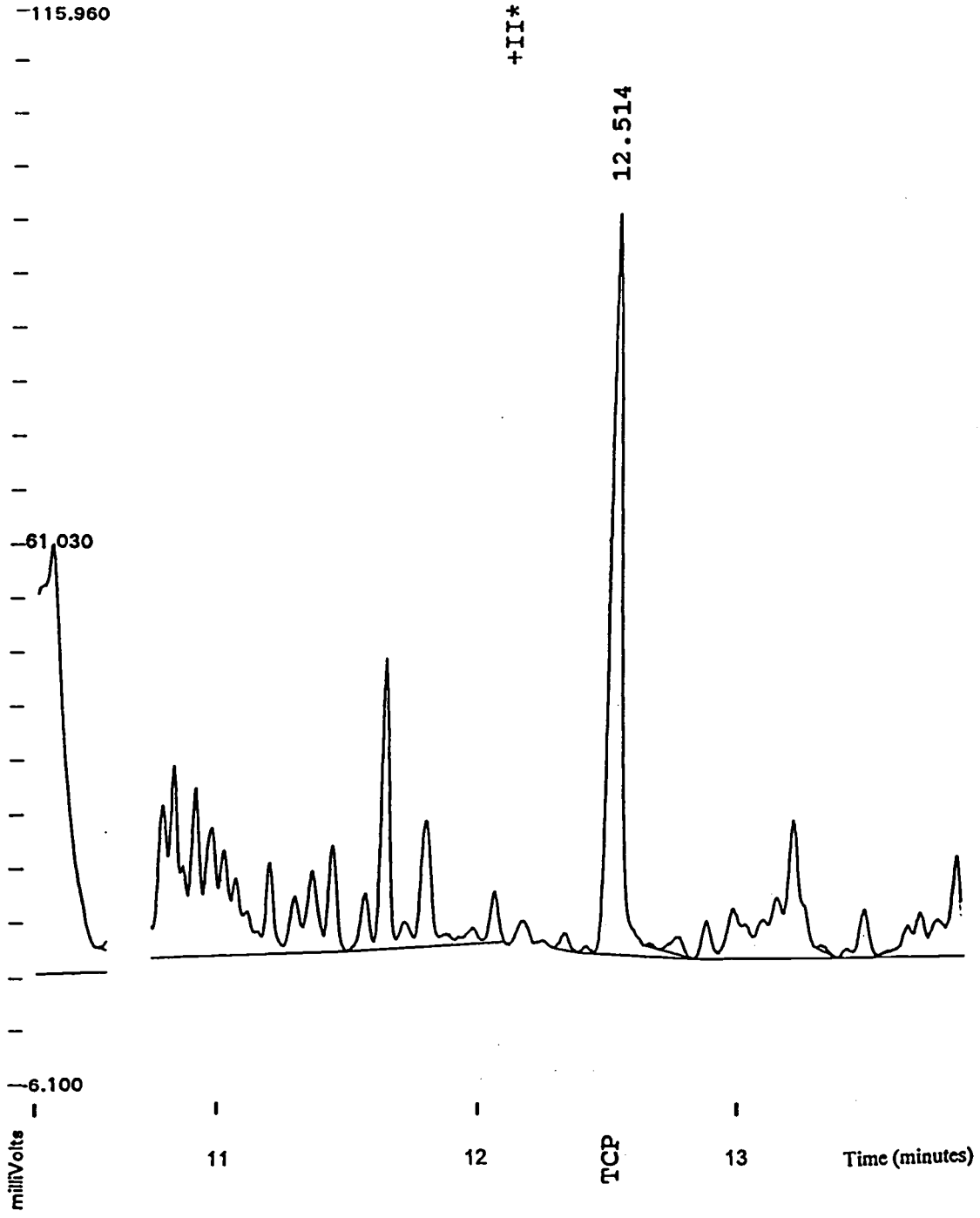

Figure 3.16a: Chromatogram of 10ng/mL TCP standard (attenuation 50)

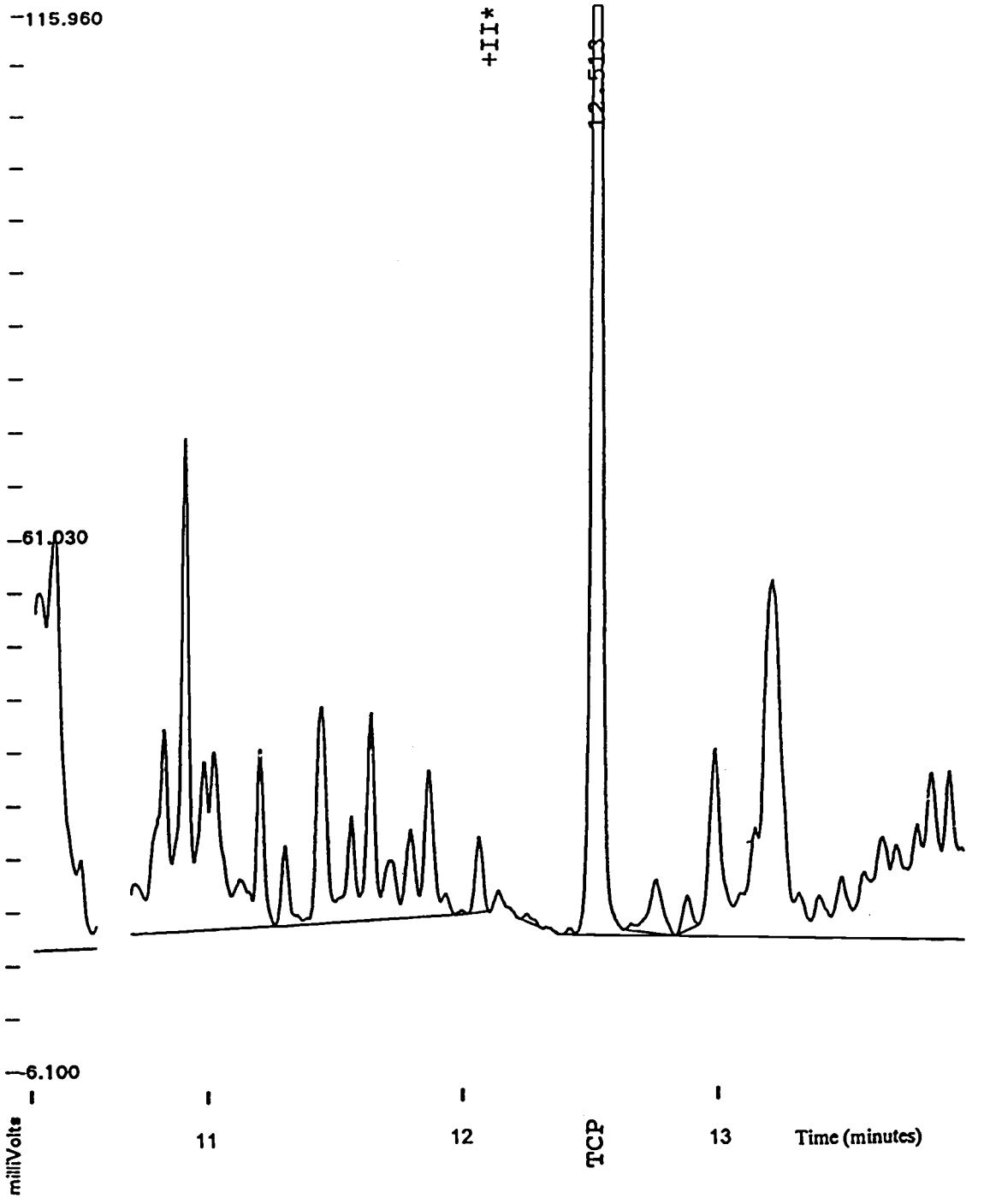

Figure 3.16b: Chromatogram of blank water spiked with 49 ng of TCP (attenuation 50)

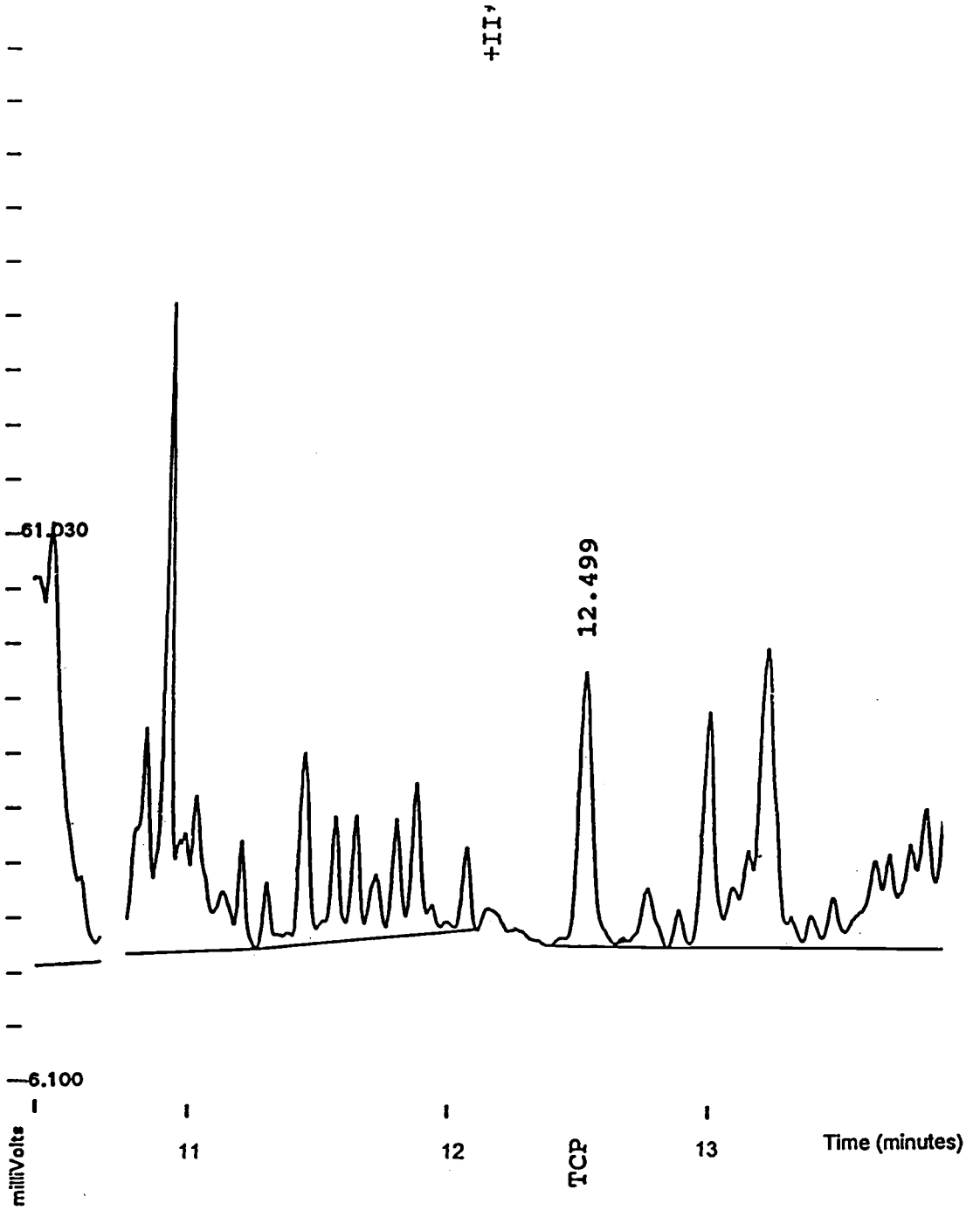

Figure 3.16c: Chromatogram of drinking water (from well 1) analysis for TCP (attenuation 50)

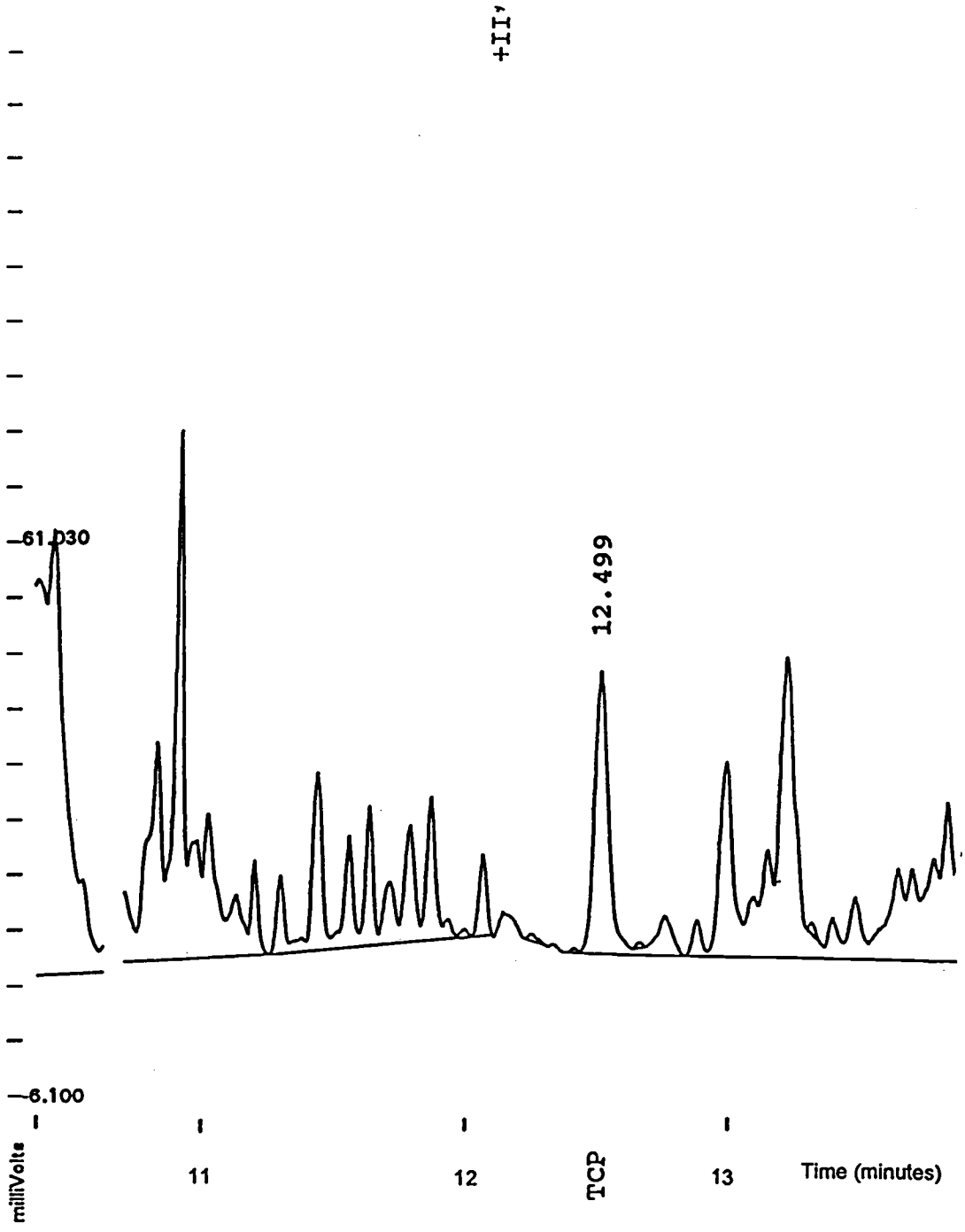

Figure 3.16d: Chromatogram of drinking water (from well 2) analysis for TCP (attenuation 50)

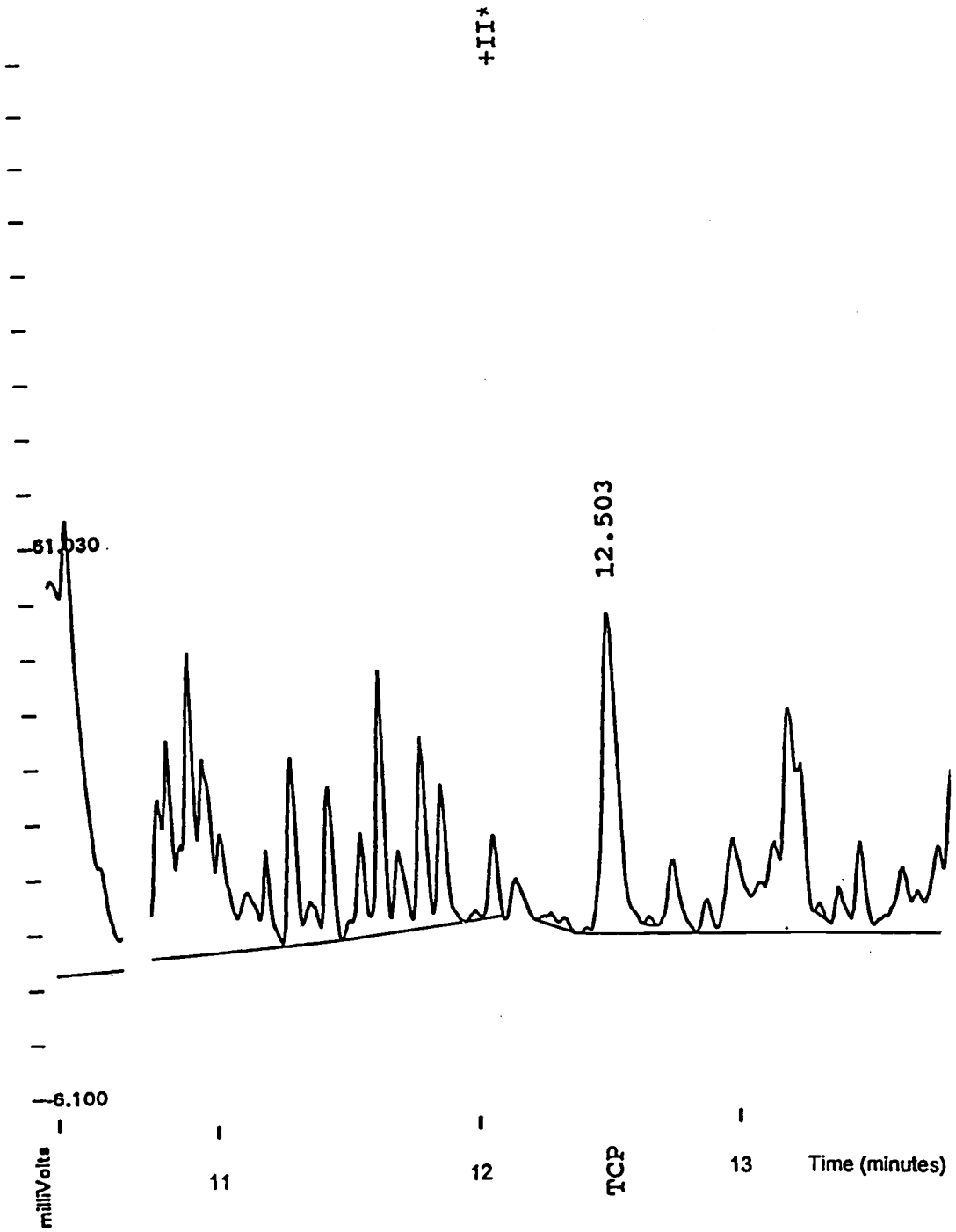

Figure 3.16e: Chromatogram of drinking water (from well 3) analysis for TCP (attenuation 50)

DISCUSSION

Assuming the source of TCP in well water is contamination from spray operation, and one TCP mole was generated from one mole of chlorpyrifos, the average TCP level is equivalent to 12.69 ng/mL of chlorpyrifos in water. Assuming the parent was present and assuming a 70 kg adult drink 2L of water a day, the internal dose by drinking water would be 0.36 ng/kg/day. RfD for chlorpyrifos is 0.003 mg/kg/day, and, hence, hazard quotient and margin of safety of drinking water is 0.121 and 83.3, respectively. One would conclude that any contribution from water or house dust to the farmer's overall exposure would be minimal. This exposure would not represent a significant increment for the farmer over and above the exposure (0.0021-0.0084 mg/kg) he received during application of the chlorpyrifos.

One might have expected to detect more chlorpyrifos in wells located in the middle of the treated fields. However, the areas treated were relatively small, and the back-pack sprayers would not produce aerosols susceptible to drift. In addition, high soil moisture and pH (~7) would enhance degradation. It is most likely that the TCP leached into the well after hydrolysis of the chlorpyrifos in the soil.

Failure to detect chlorpyrifos in house dust is not consistent with observations in the USA (Davis and Ahmed, 1998; Gurunathan et al., 1998). This distinction may also reflect differences in areas treated and persistence. The farmers in Sri Lanka treat only small area in contrast to the many acres that may be involved in a larger operation. With higher soil temperatures in tropical areas and a soil pH of about 7, the chlorpyrifos would be less persistent than in temperate zones.

CONCLUSION

Chlorpyrifos was not present with detection limits of 7 ng/L in drinking water wells located near fields treated with this organophosphate. Small quantities of trichloropyridinol metabolite (9, 10, and 0.6 ng/mL) were detected in well water. House dust collected in houses close to treated fields did not contain chlorpyrifos. Neither well water nor house dust contributed to the farmers' exposure to chlorpyrifos.

CHAPTER 4

CONCLUSION

Farmers applying chlorpyrifos showed a consistent excretion pattern of the metabolite, TCP, characteristic for this organophosphate. The excretion half-life ranged from 24.8 to 37.6hr with an average value 31.3hr. The cumulative TCP excreted over 120hr was used to calculate the internal dose of chlorpyrifos, which ranged from 0.0021 to 0.0084 mg/kg. It was assumed that major exposure route was skin, and a dose of 0.4 to 1.2 mg/kg was estimated, based on 1% dermal uptake. This dose was considered to give a marginal risk with hazard quotients range from 0.7 to 2.7 and margin of safety from 4 to 14. Statistical analysis established that the internal dose was determined, in large part, by the amount of chemical applied. In addition, it was demonstrated that faulty spray equipment and the amount of skin exposed also was associated with an increase in the internal dose. Analysis also indicated that wearing long pants could increase the internal dose, although the reason for this unexpected response is not clear. This study provides quantitative information for that program, which can be used to train farmers in the use of safer application practices.

Chlorpyrifos was not present with detection limits of 7 ng/L in drinking water wells located near fields treated with this organophosphate. Small quantities of trichloropyridinol metabolite (9, 10, and 0.6 ng/mL) were detected in well water. House dust collected in houses close to treated fields did not contain chlorpyrifos. Neither well water nor house dust contributed to the farmers' exposure to chlorpyrifos.

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APPENDIX

APPENDIX

QUESTIONNAIRE FOR FARMERS

Appendix

Serial No:

Weight:

Height:

Division of Health Service:

1) Name:
2) Address:
3) Educational Qualifications:
    a) Up to 5 years
    b) Up to year 6- 8
    c) Year 8 or above
4) Occupation:
    a) Fulltime spray operator
    b) Fulltime farmer
        i) Self application of pesticides
        ii) Applicator is not the farmer
    c) Part time farmer:
        i) Self application of pesticides
        ii) Applicator is not the farmer

5) Using integrated pest management systems: Y/N

6) Number of family members:

a) Less than 1 year
b) Between 1-5 years
c) Between 5-12 years
d) Between 12-18 years
e) Between 18-40 years
f) Over 40 years

7) Pregnant woman:

8) Pesticides are used on :
a) paddy
b) vegetables
c) Other

9) Cultivation:
a) Seasonal
b) Throughout the year

10) Days between last application and harvest:

11) Frequency of pesticide application:
a) Hours per week
b) Tanks per week
c) Land area

12) Distance to the field from the house

13) Distance to the closest agricultural land from your house

14) Time of pesticide application: start and end

15) After applying pesticides:

a) Bathing — After how long

b) Washing hands, legs, face — After how long

16) Amount of concentrated pesticides per tank:

17) Pesticides used during the past week:

Pesticide	Method of application	Concentration

18) Does anyone help you apply pesticides:

If yes, who

19) When applying pesticides:

a) Do you use alcohol

b) Chewing beetles

c) Other food

20) Safety measures used when applying pesticides

a) Face cover

i) Face mask

ii) Face cover

iii) Handkerchief

iv) Other face cover device

b) Banian

c) Shirt — Long-sleeved — Short-sleeved

d) Pants — Long — Short

e) Saron — Up to the knee — Full

f) Gloves

g) Sleepers/ Shoes

21) Weaknesses in application of pesticides:

a) Tank is leaking

b) Damaged gloves

c) Damaged shoes

d) Damaged clothing

22) Spray tank:

Good condition

Leaking

Blocked nozzles

Cleaning the tank

What time

Who

Where

23) Pesticide storage at home:

a) Kitchen

b) Roof

c) Field

d) Garden

e) Other

24) Source of drinking water

a) Tap

b) Tube well

c) Well

d) Stream

25) Distance between source of drinking water and closest field:

Less than 10m

Between 10-20m

More than 20m

26) Alcohol consumption

Daily/ occasional

27 Smoking: how many

26) Health related problems in the family:

a) Children: yes no

b) If no children:

i) Married for how long:

ii) Ages of male and female:

iii) Number of years pesticides applied:

c) Are there any other married family members without children:

If yes who:

27) Are you or any your family members suffering from following diseases:

a) Cough

b) Short of breath

c) Asthma

d) Angina pectoris

e) Palpitation

f) Faintness

g) Swelling of ankle

h) Nausea

i) Vomiting

j) Loss of weight

k) Constipation

l) Diarrhea

m) Abdominal pain

n) Dysuria

o) Polyuria

p) Urinary incontinence

q) Urgency

r) Muscle ache/mayalgia

s) Arthalgia

t) Arthritis

u) Headache

v) Visual defects

w) Hearing defects

x) Adomnia

y) Giddiness

z) Stammering

aa) Dysphagia

ab) Ataxia

ac) Loss of consciousness

ad) Numbness

af) Shivering

ag) Itching

ah) Burning sensation of the eye

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ANALYSIS OF WATER FOR PESTICIDES IN TWO MAJOR AGRICULTURAL AREAS OF THE DRY ZONE

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ABSTRACT

Water samples from irrigation tanks and drinking water sources in Polonnaruwa and Dambulla areas of Sri Lanka were tested for chlorpyrifos, dimetoate, propanil, captan, and diazinon. Polonnaruwa was chosen for the study to represent major paddy-growing districts in the Dry-zone while Dambulla represented areas of intensive pesticide use on upland crops. Eighteen tanks and twelve drinking water wells were selected for sampling at monthly interval basis for six times during *yala* 2002 and *maha* 2002/3. Out of 544 samples analyzed, 8 were positive for either chlorpyrifos (levels ranged from 0.022– 0.542 µg/l) and/or diazinon (levels ranged from 0.012-0.15 µg/l) and one sample consisted of 0.014 µg/l of dimethoate. A multi-residue extraction method using gas chromatography was developed for analysis. The absence of residues in the water sources may have been due to enhanced dissipation caused by hot weather conditions, higher radiation, basic pH and organic carbon in water prevailing in the areas and unavailability of long persistent pesticides. Surveys conducted on pesticide usage and related adverse health effects revealed that farmers take minimal precautions when handling and 70% do not apply recommended dosage. About 21% of the farmers have suffered from acute toxicity symptoms such as dysuria, myalgia, and headache.

KEYWORDS: Pesticides exposure, Water, Health, Analysis.

INTRODUCTION

Pesticides are defined as any substance intended for use or used to control any organism posing adverse effects to human welfare. In Sri Lanka, insecticides are mainly used to control agricultural pests and malaria vectors, where as herbicides and fungicides are mainly used in agriculture. Use of pesticides on agriculture is considered as an efficient method of controlling pests, but application of these substances poses a direct risk to non-target species including human, domestic animal, wildlife and to the environment.

In agriculture, pesticides are either directly incorporated into the soil as a solid or sprayed on to the crop. Eventually, a portion of these toxins or their metabolites ends up in soil or water. In addition, pesticide spray equipments are washed in streams located closed to the farms. Racke, 1993 described that much of the chlorpyrifos applied to foliage eventually reaches soil either as parent compound or metabolite. Re-deposition of atmospheric chlorpyrifos (Racke, 1992) and spills during storage, transportation, mixing or cleaning

spray equipment could also contribute to soil levels of chlorpyrifos. Formulation and repacking of pesticides, pest control operations, disposal of pesticide water, mosquito control programs are some of the other activities that contribute to contaminate soil, air and the water. Rain, irrigation and wind carry pesticides and residues into various components of the ecosystem, which includes ground water, surface waters, tanks, lakes, and different layers in the soil. Contaminated food, water, and soil pose an indirect risk to none targets such as human.

Giesy *et al.* (1999) studied the chlorpyrifis concentration in surface water of different locations of the Lake Erie drainage basin watersheds. The levels ranged 39-2828 ng/l in the samples collected during 1987-1995. Goh *et al.* (1990) analyzed the levels of methamidophos and profenofos levels in green leafy vegetables offer for sale in Singapore. He reported that 2.4-31.7 ppm of methamidophos and 1.1-5.4 ppm of profenofos in the collected samples.

It has been estimated that 95% of fatal pesticide poisonings occur in developing countries, many of which are in the Asia-Pacific region. In Sri Lanka, the number of hospital admissions due to organophosporus (OP) pesticide poisoning in 1992 was 11,439, which was 73% of total pesticide poisonings (Fernando, 1995). Death records indicated that OPs are the major case of pesticide poisoning (Fernando, 1995; Senanayake and Peiris, 1995). Most of these poising cases are suicidal attempts, but not occupational illnesses. In another study, Jayaratnam (1987) indicated that 5 out of 1000 agricultural workers in Sri Lanka were hospitalized yearly due to pesticide poisoning from occupational exposure. But, due to the lack of a well-established medical surveillance system in Sri Lanka, most occupational poisoning cases are discharged from the hospitals without recording. A study performed among vegetable farmers in three cultivating regions in Sri Lanka indicated that 63.5% of the farmers use more than the recommended dose of pesticides, 85.7% applied pesticides before the appearance of pests, and 8% sprayed pesticides prior to marketing (Chandrasekera *et al.*, 1985). Aponso *et al.* (2002) reported that farmers occupationally exposed to chorpyrifos had only a marginal risk.

Agricultural activities in the dry zone areas depend on irrigation water supplied from internal water bodies such as tanks. Due to the scarcity of water, residence use tank water as the major source of water for all activities including drinking. Pesticides are extensively used on crops such as onion and chillie due to higher susceptibility to pest and diseases and relatively high economic returns from these crops. Therefore, water tanks and other water sources in Dambulla area where such crops are extensively grown are at a greater risk of contamination with pesticides. Polonnaruwa is one of the major rice growing districts in Sri Lanka. Applying pesticides on low land crops

such as rice would have a greater chance of contamination down stream and tank water. Since tank water is not only a source of irrigation water, but also an important resource to the daily needs of the community, consumption of tank water would pose a health risk for the residents.

Pesticide usage statistics in Sri Lanka indicate that about 60% of total insecticides were OPs. Major OP's used in agriculture are chlorpyrifos 40% emulsifiable concentrate (EC), dimethoate 40% EC, and diazinon 5% granules (G). Quantities of formulated products used during 2001 were 180, 133, and 278 metric tons, respectively. Usage of the major herbicide propanil, and fungicide captan were 552 and 18 MT respectively during the year 2001. Pesticide usage statistics in y*ala* season of 2001 is given in table 1 (Registrar of Pesticides, 2002). This study focused on measuring the tank and drinking water for possible contamination of major pesticides used in the dry zone of Sri Lanka, to assess the potential adverse health effect to the human and risk to the environment.

MATERIALS AND METHODS

Selection of areas and tanks for the study

Polonnaruwa and Dambulla area were selected for the study to represent rice and vegetable growing areas of the dry zone. When selecting tanks, more emphasis was paid to represent the worst-case scenario of the dry zone. Most of the tanks selected had paddy fields as catchments and hence high potential of contamination is anticipated. Tanks selected and locations of the drinking water sources selected for the study are given in table 2.

Pesticides selected for the study

Pesticide usage in Polonnaruwa and Dambulla areas were obtained from the pesticide marketing companies before selecting pesticides for the study. Toxicological and other scientific information of all pesticides (table 3), quantities used in each area, available analytical facilities were considered when selecting pesticides.

Sample collection

Samples were collected in four to five week intervals throughout both seasons. Four representative sampling points were identified from each tank. Three replicates were collected from each sampling point and an aggregated sample from one sampling point was analyzed due to the time constraint and limitation of resources.

Table 2. Tanks selected and locations of drinking water sources.

	Polonnaruwa	*Dambulla*
	Tanks selected	
1	Borawewa	Rangirigama wewa
2	Ellewewa	Palutawa wewa
3	Kasanwewa	Tharana Wewa
4	Mahawanawala	Titthayakola wewa
5	Nagaswewa	Kandalama Wewa
6	Parackrama Samudraya	Saluappulana Wewa
7	Paranagama wewa	
8	Medagama wewa	
	Drinking water collected sites	
1	Aralaganwila	Batuyaya (agrowell)
2	Borawewa	Rangirigama (agrowell)
3	Kasan wewa	Tharana wewa
4	Mahawanawalawewa	Titthayapola wewa
5	Medagama	Karawilahena
6	Polonnaruwa	Galewela

Table 3. Details of pesticides Selected for the study and toxicological Information of each compound.

Pesticide	*CAS RN*[#]	*ADI mg/kg b.w*	*NOEAL*[%] *mg/kg b.w.*	*Toxicity*		*Reasons for the selection*
				LD_{50} (mg/kg) Rat Oral	*Hazard Class*	
Chlorpyrifos	2921-88-2	0.01*	0.03	135-163	II	Widely used, Broad spectrum
Dimethoate	60-51-5	0.002*	5.0	387	II	Widely used, Broad spectrum
Propanil	709-98-8	0.005[+]	400	>5000	IV	Widely used, higher volume per acre
Captan	133-06-2	0.1*	2000	9000	III	Widely used fungicide
Diazinon	333-41-5	0.002*	0.06	2000	III	Widely used, applied to soil

[#] CAS RN Chemical Abstract Service Register Number

* JMPR Joint meeting of the FAO Panel of Experts on Pesticide Residues and the Environment and the WHO Expert Group on Pesticide Residues (Pesticide manual 1999-2000)

[+] US United States environmental program

[%] *2 year studies using rats*

b.w. Body weight

Survey on adverse health effects of pesticides used in the dry zone

A questionnaire was completed for fulltime farmers selected from both areas. About 25 farmers from each area participated in the study. Personal and agricultural details of each farmer were recovered prior to the health status assessment. Two specialist doctors (neurology and gynecology) from Faculty of Medicine, University of Peradeniya, examined acute and chronic toxicity signs and symptoms of the farmers.

Multi-residue extraction method to extract chlorpyrifos, Dimethoate, Diazinon and Captan from water

Multi-residue analytical method was developed using the methods described by Aponso *et al.* (2001) and USEPA (1994). A sub-sample of 200 ml was acidified with 1 ml of 10N HCl and extracted with dichloromethane (2 x 10 ml aliquots) and hexane (1x10ml) in separatory funnels. All extracts were combined and dried with 3-5g of anhydrous sodium sulphate. Then the total volume was concentrated to 1 ml under nitrogen gas for injection on to the gas chromatograph (GC) fixed with an electron capture detector (ECD). Recovery of chlorpyrifos, dimethoate, diazinon and captan from spiked water was 89, 72, 75 and 70 % respectively. Detection limits for chlorpyrifos, dimethoate, diazinon and captan from water were 51, 0.86, 203, and 58 ng/l respectively.

Extraction Method for Propanil

A sub-sample of 200 ml was acidified with 1 ml of 10N HCl and extracted with toluene (2 x 20 ml aliquots) in a separatory funnel. The extracts were combined and dried with 3-5g of anhydrous sodium sulphate. Then the total volume was concentrated to 1 ml under nitrogen gas for injection into the GC. Method described by Zweig and Sherma (1984) was modified as relevant for this analysis. Recovery of Propanil was 70% from spiked water. Detection limit for propanil in water was 52.9 ng/l.

Gas chromatographic conditions

A HP 5890 gas chromatographic system with electron capture detector and a HP 35 column was used. Temperature at injector, and detector ports were 225 and 250 ^{0}C, respectively. Three-step column oven temperature program of 110, 190, and 275^0 C for 1, 10, and 2 min, respectively employed for analysis. The rates of temperature increases were 110 to 190 at 30 ^{0}C/min and 190 to 250 at 20 ^{0}C/min.

RESULTS

Analysis of the questionnaire

Survey results show that most of the farmers take minimal precautions when applying pesticides, which enhances dermal absorption. Most of them live close to an area where pesticides are used. Sources of drinking water of the residents are also located in the cultivated fields or an adjacent location. About 70% of the farmers do not use the recommended concentration when applying pesticides. Fifteen of them (out of 17) use higher doses than recommended.

Medical examination

It was noted, from medical symptomatology, that 83% of the farmers had symptoms related to acute toxicity, but 21% of the group surveyed confirmed as having effects that are related to exposure to pesticides. Three main possible acute effects of pesticide poisoning found in the survey are dysuria, myalgia, and headache.

Results of the water analysis

a) Water from Polonnaruwa

pH of tank water ranged 6.81- 8.18 (average 7.5) and all samples consist of organic matter as suspension. Figure 1 shows the levels of pesticides found in water samples collected both from Polonnaruwa and Dambulla during *maha* season 2002/2003.

Borawewa

Borawewa is the major source of water for the residents of this area. Most of the drinking water wells dry up during drought periods and hence the tank water is the only source of water for the residence. A sample collected prior to the cultivation season had 0.0082 µg/l of chlorpyrifos, which was unusual. Another sample collected close to the spill of the same tank, six weeks after the start of cultivation had 0.20 µg/l of diazinon (figure 2), but none of the other water samples collected from this tank or drinking water well in Borawewa had quantifiable levels of any of the pesticides tested.

Ellewewa

Ellewewa tank is the major source of drinking water for the area. None of the samples collected from this tank had pesticides residues.

Kasanwewa, Mahawanawalawewa and Nagaswewa

Water of these tanks are used for agriculture and drinking purposes. Samples collected close to paddy fields of these tanks had detectable levels of diazinon, six weeks after cultivation. Levels found were 0.01,0.55, and 0.085 μg/l for Kasanwewa, Mahawanawalawewa and Nagaswewa respectively (figure 2). The drinking water collected from Mahawanawalawewa had 0.052 μg/l of dimethoate at the same timing.

Paranagama wewa

A sample collected, six weeks after cultivation, close to the spill of the tank had 0.085 μg/l of chlorpyrifos, but none of the other water samples collected form the tank had quantifiable levels of any of the pesticides.

Medagama wewa

The dinking water sample collected in the sixth weeks after cultivation had 0.109 and 0.032 μg/l of chlorpyrifos respectively (figure 4).

b) Results of Dambulla

None of the water samples collected from Dambulla had detectable levels of the considered pesticides.

DISCUSSION AND CONCLUSION

Out of nearly 306 samples analyzed from the Polonnaruwa area, only 8 (2.6%) were detected with pesticide residues. The levels detected for diazinon and chlorpyrifos were below 0.55 μg/l and 0.109 μg/l respectively. Severe drought conditions prevailed during the months of November and December 2002 (six weeks after cultivation), water levels in the tanks were low and hence the concentration of the dissolved matter was expected to be higher than usual. About 234 samples were analyzed from Dambulla, but none of them were found contaminated with pesticides analyzed for. Out of the five pesticides tested, only three were found in water.

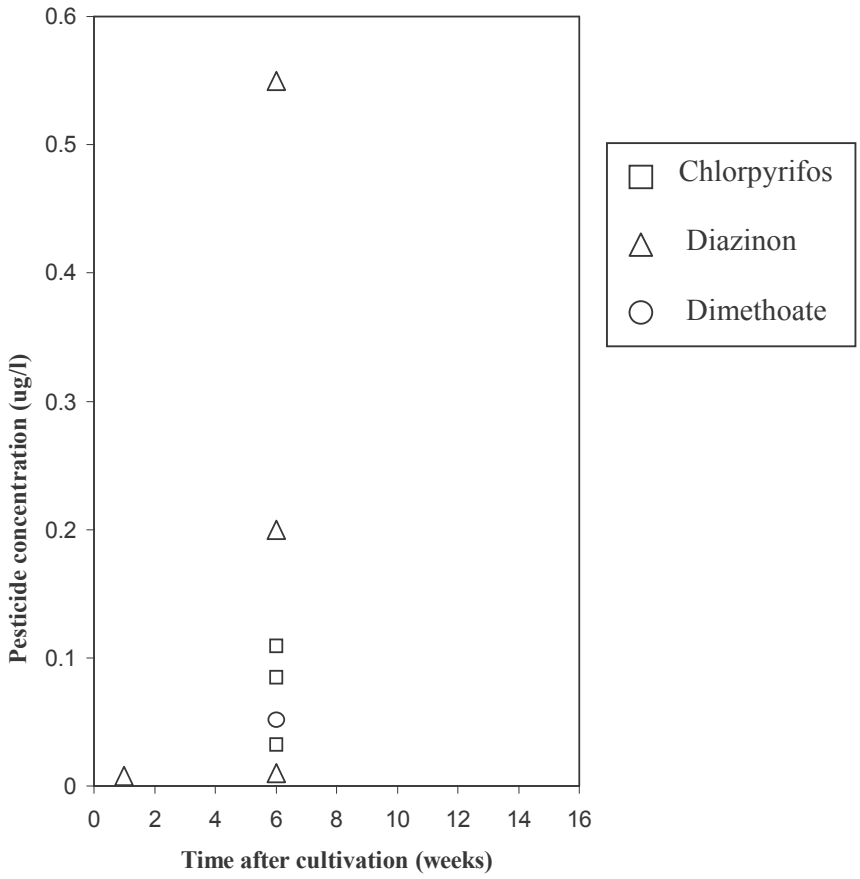

Figure 1. Pesticide levels detected in water samples collected from Polonnaruwa and Dambulla during *maha* season 2002/03 with time after cultivation.

There are three major aspects as identified below, that could have contributed to the absence of pesticide residues in most of the samples analyzed, in spite of number of malpractices found with farmers when using pesticides.

1. Intrinsic properties of the chemicals
2. Environmental parameters
3. Practical field situations

Pesticide formulations are normally diluted nearly 700 fold with water prior to spray applications. For example, a typical spray mix of chlorpyrifos contains 500 ppm of the chemical. It is normally sprayed directly onto the crop as a thin layer of spray mist and only a small fraction of the chemical would reach the ground.

In Polonnaruwa, the submerged conditions are relatively less compared to wet zone paddy cultivation. Therefore, down stream transport of pesticide residues to the tanks is relatively inefficient. In upland crops such as vegetables grown in Dambulla pesticides such as chlorpyrifos, dimethoate, diazinon and captan are more frequently used than on rice. When pesticides are applied on upland crops they essentially end up in soil. There must be rain or irrigation that would carry the chemical to an aquatic system. Depending on the lipophilic properties of the product a portion of the chemical would be bound to the organic matter in the soil. Thus the availability of the chemical to contaminate aquatic sources is further restricted.

When tanks are considered, though the rate of contamination is higher compared to the ground water, due to physical factors such as run-off. However, the dilution rate is extremely high, because of the large size of the water body. Once it reaches the aquatic environment abundant UV radiation and, relatively high water temperature in the dry-zone, combined with basic nature and high organic matter content in the water enhance the rate of degradation. Normally, it is recommended that pesticides should be applied on sunny days, which further facilitate the rapid degradation.

Positive and pragmatic approaches in policy decisions of regulation of pesticides in Sri Lanka may also have contributed to the favorable situation observed in the current study. None of the pesticides available in Sri Lanka are long persistent in the environment; therefore, bioaccumulation or biomagnification is unlikely. Further the banning of extremely and highly hazardous pesticides in Sri Lanka in mid nineties may have contributed to absence of severe acute exposure health effects in the farmers.

However, unwarranted practices such as washing spray equipments in streams, disposal of empty containers close to water bodies would have a higher potential to contaminate internal water bodies such as water wells and small tanks. Applicators are continuously exposed to low concentrated mixture while applying on crops. Applicators are at a higher health risk due to long hours of exposure while spraying and due to minimal safety precautions taken during handling concentrated pesticides. The water analyses results suggest that exposure potential from water sources is less significant for the farming communities in the dry-zone. According to the survey conducted for pesticide applicators of the study area, very high prevalence has been recorded for use of pesticides more than the recommended dosages. Further there are strong indications of acute pesticide poisoning potential among the farmers. These findings suggest that the level of misuse of pesticide contributes greatly to occupational exposure than the environmental risk.

Survey information collected from the community of the area on health aspects clearly indicated that there are no signs or symptoms due to acute pesticide exposure. Potential health risk of exposure to the highest levels of chlorpyrifos residues found in the water sources, if extrapolated for an average adult of 70 kg bodyweight who would intake 2 l of water per day from Medagama wewa, which had the highest level of chlorpyrifos (0.109 µg/l), his daily intake is 0.218 µg of the active ingredient. The Acceptable Daily Intake (ADI) established by the JMPR (FAO/WHO) for chlorpyrifos for human is 0.01mg/kg body weight, which is equivalent to 0.7 mg for a 70 kg person. Similarly, the highest diazinon and dimethoate exposure through water consumption from sources subjected to the study were 1.1 µg and 0.104 µg per day respectively. ADI for both of those compounds is 0.14 mg, when extrapolated to an average adult. These findings suggest that the level of exposure to pesticide residues in the study area is significantly lower than that of the internationally accepted ADI. Therefore, it is unlikely to have acute adverse health by consuming water from the tanks in the study area, which confirms the observations of the health survey conducted.

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EFFECT OF APPLIED Zn ON N USE EFFICIENCY, GROWTH AND GRAIN YIELD OF RICE GROWN IN LOW HUMIC GLEY SOILS OF LOW COUNTRY INTERMEDIATE ZONE

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ABSTRACT

Zinc (Zn) is the most important micronutrient that limits the rice yield in Sri Lanka at present. Its deficiency in soils would reduce the use efficiencies and recoveries of other essential nutrients affecting the rice yield. Therefore, a two factor factorial experiment was conducted for two seasons using two levels of Zn (viz. 0 and 2.5 Kg Zn/ha) and 5 levels of nitrogen (viz. 0, 75, 100, 125 and 150 kg N/ha) with recommended levels of P and K to study the effect of Zn on N use efficiency, recovery, growth and grain yield of rice grown in Low Humic Gley (LHG) soil in Low country Intermediate Zone of Sri Lanka. Soil analysis reveals that soil was deficient in Zn and application of Zn at the rate of 2.5kg Zn/ha induced the N use efficiency from 15.6 to 19.4 kg grain yield per kilogram of applied nitrogen and N recovery from 31% to 41% by rice and augmented higher rice yield in LHG soils. A combination of 100 kg N and 2.5 kg Zn gave the same yield as that of 125 kg N/ha alone. The interaction between N and Zn on grain yield was synergistic. Residual effect of Zn on the same was also observed in the second crop of rice.

KEYWORDS: LHG soils, Applied Nitrogen, Zinc use efficiency, Rice yield.

INTRODUCTION

Annual extent of rice cultivation in Sri Lanka is 0.89mha (Abeysiriwadana and Sandanayaka, 2000). Ninety eight percent of this area is cultivated using new improved rice varieties, which require high level of fertilizer application to obtain maximum yield while sustaining the soil fertility. Annual estimated requirement of fertilizer is 0.32 million metric tons and this entire requirement is annually imported to Sri Lanka incurring a cost of 2.88 billion rupees (NSF, 2000). On the other hand, fertilizer N use efficiency by the rice plant under the present system of cultivation in Sri Lanka is estimated to be around 25-30% and the rest is lost in the rice ecosystem due to improper soil fertility management in paddy soils (Sirisena *et al.*, 2001a). This situation has led to use of large amount of foreign exchange while potentially polluting the environment as well.

Nutrient imbalance in soils produces low fertilizer use efficiency, low yields and low farmer profit (Tiwari, 2002). It also results in further depletion of the most deficient nutrients in the soil. Once a nutrient is reached to its

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Environmental Toxicology

SEASONAL EXPOSURE OF FISH TO NEUROTOXIC PESTICIDES IN AN INTENSIVE AGRICULTURAL CATCHMENT, UMA-OYA, SRI LANKA: LINKING CONTAMINATION AND ACETYLCHOLINESTERASE INHIBITION

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Abstract—The annual cultivation pattern in the Uma-oya catchment in Sri Lanka is characterized by *Yala* and *Maha* rainfall periods and associated cropping. Two cultivation seasons were compared for pesticide residues: base flow, field drainage, and the runoff and supplementary sediment data for three sites in the catchment. Organophosphate and N-methyl carbamate pesticide analysis confirmed a higher concentration in the *Yala* season with low-flow conditions. Acetylcholinesterase (AChE) activity was measured by standard spectrometry in the brain, muscle, and eye tissues of three freshwater cyprinid fishes, *Garra ceylonensis*, *Devario malabaricus*, and *Rasbora daniconius* from three study sites during months overlapping two seasons in 2010 (December) and 2011 (July). Baseline AChE data were measured from fish samples from a forested reserve in the Knuckles. A 73% inhibition in muscle AChE activity in *G. ceylonensis* was associated with intense pesticide exposure months in the *Yala* season. The AChE inhibition more than 70% in *G. ceylonensis* eyes in both *Yala* (76%) and *Maha* (72.5%) seasons indicates particular sensitivity of eye tissue to inhibitors. The less dramatic AChE inhibition in the eye tissues in *D. malabaricus* and *R. daniconius* in both seasons indicates exemplary protective capacity of muscle AChE in fish. The highest inhibition of AChE (up to 60% in brain and up to 56% in muscle AChE activity in *R. daniconius* and up to 47.8% in brain and up to 64.6% in muscle AChE activity in *D. malabaricus*) occurred during the *Yala* season. Tissue AChE activity and physiological activity in fish were correlated. The results collectively indicate that AChE is a consistent biomarker for diffused contaminant exposure in agricultural catchments. Environ. Toxicol. Chem. 2012;31:1501–1510. © 2012 SETAC

Keywords—Agricultural catchment Acetylcholinesterase Cyprinid fish species N-methyl carbamates Organophosphates

INTRODUCTION

The use of pesticides in developing countries has increased as the scale of cropping has expanded [1], and at the same time, pesticide use has been increasing without a major change in agricultural land use [2]. Sri Lanka is endowed with monsoonal climates that favor seasonal shifts in cropping patterns. Pesticide input into associated aquatic ecosystems is driven by both hydrological events and agricultural practices. Contemporary pesticide classes such as organophosphates (OPs) and N-methyl carbamates (NMCs) are short-lived in the environment, and their use has increased markedly in developing countries after the banning of highly persistent organochlorines [3]. The major distinction between the two classes of insecticides is the duration of acetylcholinesterase (AChE; EC 3.1.1.7) inhibition. Organophosphate-induced inhibition is effectively irreversible, whereas inhibition by NMC is reversible, which leads to a faster rate of recovery from enzyme inhibition [4]. In 2008, cholinesterase inhibitors such as OP and NMC insecticides made up almost 88% of the total amount of insecticides sold in Sri Lanka (Office of the Registrar of Pesticides, Sri Lanka).

These contemporary compounds degrade rapidly in the environment and require frequent application, thus increasing the cumulative environmental burden [5], but they generally lack target specificity and have high acute toxicity toward many nontarget vertebrate and invertebrate species [6]. Some of the OPs and NMCs, such as chlorpyriphos, diazinon, and carbofuran, are detected more frequently in water bodies associated with agricultural fields, despite the rapid field dissipation and dilution of these insecticides [7,8]. Many of these pesticides degrade quickly under hot tropical climatic conditions, and residue from most commonly used pesticides may not be detectable in water [7].

Aquatic organisms are able to respond to contaminants even when such substances are not detectable in water or sediments [9]. Pesticides produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes, and AChE inhibition has been instrumental as a sensitive biomarker of the effect of OP and NMC insecticides in aquatic ecosystems [10,11] (see Supplemental References).

It has been suggested that AChE activity is highly conserved in organisms including fish [12], and environmental contaminants can upregulate and downregulate this enzymatic activity. From the standpoint of functionality, both effects are deleterious for fish survival [13,14]. The inhibition of activity can also affect a variety of other life functions including growth, survival, feeding, and reproductive behavior of fish [13,14], leading to population-level effects [15]. However, AChE inhibition does not necessarily lead to the death of organisms but is useful as an exposure biomarker for sublethal concentrations of contaminants [8,16] (see Supplemental References).

Several studies have shown that fish inhabiting natural freshwater ecosystems may be affected by the intentional and unintentional spreading of pesticides [9,10,17]. In vivo exposure of fish species to AChE inhibitors led to differential

All Supplemental Data may be found in the online version of this article.

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response patterns of AChE activity in different tissues (brain, muscle, plasma, liver, and eyes; [18]). Currently, qualitative differences in AChE enzymatic activities among fish species are largely associated with individual exposure conditions [14]. Comparison of differential tissue responses to AChE inhibitors among multiple species in real agricultural environments is limited [17]. Fish species with consistent and pronounced AChE activities may represent more sensitive sentinel species [19]. In addition, it is important to understand the seasonal impact of agricultural effluents that affect the physiology of the resident fish species. Previous studies reported that *Rasbora caverii*, an indigenous Sri Lankan fish species, was more sensitive to AChE inhibition compared with the exotic Mozambique tilapia *Oreochromis mossambicus* [9].

We have recently shown that *Garra ceylonensis*, *Devario malabaricus*, and *Rasbora daniconius* (Teleostei, family: Cyprinidae) are quite widely distributed in agricultural areas in Sri Lanka [20]. We designed field studies to examine the health of several species [21]. The aims of the present study were to understand the potential impacts of agricultural pesticides on native fish species by analyzing cholinergic effects on selected fish tissues and to investigate the possible correlation with known concentrations of AChE-inhibitory pesticides. The present study was conducted in the Uma-oya catchment in the upper Mahaweli River basin in Sri Lanka. We restricted our focus to OP and NMC insecticides during the assessment.

MATERIALS AND METHODS

Study area

The Uma-oya catchment is the largest in the upper Mahaweli River basin in Sri Lanka, with a mean elevation of 1,678 (range, 915–2,440 m) above mean sea level (MSL). Welimada (6°54′96″N 80°55′22″E) is one of the main townships within the catchment, with a total population of 107,369 (within the administrative division). The total discharge of the Uma-oya measured at the gauging station at Welimada was 23.4 million m^3 during the *Maha* monsoon season (October 2008 to March 2009) and 13.4 million m^3 in the *Yala* monsoon season (April to September 2008) (Mahaweli Authority, Sri Lanka). Study sites were selected from the Uma-oya (stream) and tributary stream areas where agricultural and pesticide use patterns were known. The three study stream sections were situated in agricultural villages (village 1 = Medawela, 6°56′34″N 80°50′25″E, 1,110 m above MSL; village 2 = Girambe, 6°54′50″N 80°53′13″E, 1,054 m above MSL; and village 3 = Erabadda, 6°53′00″N 80°52′38″E, 1,106 m above MSL) in Uva-Paranagama and Welimada Divisional Secretariat divisions in the middle catchment (Fig. 1); the representative catchment size was approximately 135 km^2 and the total agricultural land extent was 8,816 hectares [22]. The total agricultural area in these three villages is approximately 253 hectares of uplands and 125 hectares of lowlands (Provincial Department of Agriculture, Sri Lanka). The total rainfall during the year 2009 in the Welimada area was 1,897 mm with the highest monthly average rainfall in December (791 mm), and the lowest in June (5.8 mm). The average maximum and minimum air temperatures recorded in the area were 25.6 and 16.5°C, respectively (Department of Meteorology, Sri Lanka). Annual crops, such as vegetables and potatoes, are major land-use components that occupy the mountain slopes (i.e., uplands), valley bottoms, and plateaus (i.e., lowlands). Soil erosion is another characteristic feature in the catchment area and is caused by water runoff due to deforestation, intensive agricultural practices on fields with steep slopes (>35°), and poor handling of water resources [23].

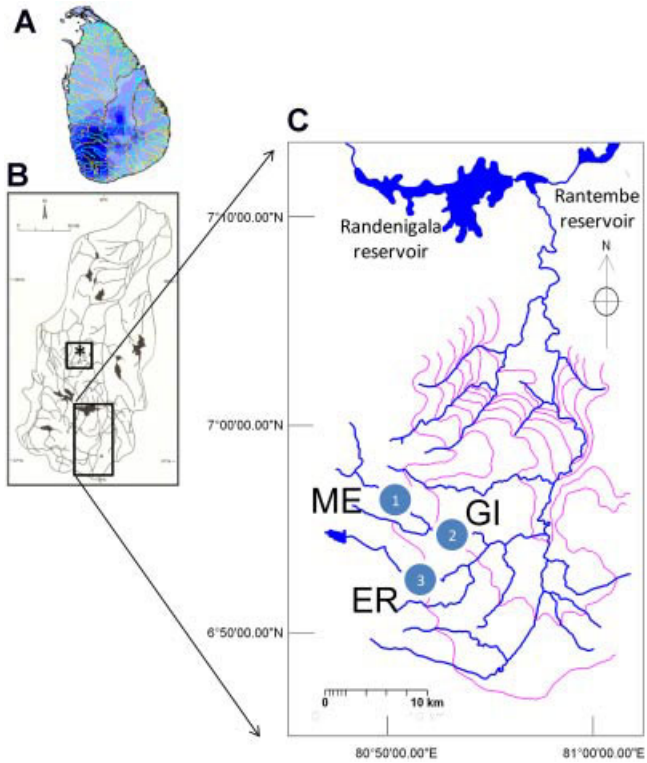

Fig. 1. Locations of three sampling stations for collection of fish, subsurface and runoff water, and sediment in the Uma-oya catchment (Upper Mahaweli River tributary, Sri Lanka). The asterisk indicates the location of the Knuckles streams for reference fish collection. (**A**) Map of Sri Lanka. (**B**) Map of stream distribution of Mahaweli River basin. (**C**) Map of stream distribution of the Uma-oya. ME = Medawela; GI = Girambe; ER = Erabadda. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

The Knuckles Conservation Forest streams (7°27′82″N 80°48′56″E) were chosen for collection of reference fish specimens (Fig. 1). Intense human activities in most of the areas were curtailed after the declaration of the area as a Conservation Forest in May 2000 by the Government of Sri Lanka. The Knuckles is covered with montane forests and an associated buffer zone of approximately 350 km^2, which spans from 200 to 1,900 m above MSL [24]. The Knuckles streams are part of the Amban-ganga catchment, which receives 1,435 to 2,111 mm of rainfall annually (as measured in six stations within the catchment during 2008–2010). A maximum air temperature of 31.5°C and a minimum of 20.2°C was recorded during 2008 and 2009 (Department of Meteorology, Sri Lanka).

Sampling strategy and storage for pesticide analysis

The target pesticides of OP and NMC classes of insecticides were selected from a list of most used pesticides in the three study villages in the catchment [25]. Field samples (water and sediment) were collected in four representative months in two different seasons: during October and December 2010 (*Maha* season) and June and August 2011 (*Yala* season), in periods overlapping active cropping in the Uma-oya catchment. To assess spatial variation in terms of contamination by pesticides, subsurface water in the mainstream and field canal, runoff water, and sediment samples were collected from the following three sites in the Uma-oya (Fig. 1): Medawela (upstream of the mainstream), Girambe (downstream of the mainstream), and

Erabadda (tributary stream) surrounded by agricultural fields. To assess the potential of runoff input from agricultural fields, samples were collected twice during major rainfall events in the *Maha* monsoon season. Sediment samples were collected using a stainless steel corer and transferred into polyethylene bags, and duplicate water samples (subsurface and runoff water) were collected in 1-L amber glass bottles following standard practices [26,27]. All samples were transported on ice in rigid-form boxes. In the laboratory, the sediment samples were air-dried, homogenized, and sieved (2-mm mesh size). Water and sediment samples were stored in the dark at 4°C (maximum storage time, two weeks) until analysis.

Pesticide analysis

The priority of pesticides were the OPs (chlorpyriphos, diazinon, dimethoate, profenophos, and phenthoate) and the NMCs (carbofuran, carbosulfan, and carbaryl). Water (subsurface and runoff in the mainstream and field canal) and sediment samples were analyzed for the presence of pesticides in the Chemical and Microbiological Laboratory of the Industrial Technology Institute, Colombo, Sri Lanka. Quality assurance and quality control of all analyses were ensured by participating in quality checking under the Swedish Board for Accreditation and Conformity Assessment (1791: ISO/IEC 17025) and the Sri Lanka Accreditation Board (ISO/IEC 17025, TL004) accreditation systems. Two manuals [28,29] were used to create a combination of test methods (previously validated) that were followed for all compounds, using a HP 5890 gas chromatograph fitted with an Agilant 7973 Auto Sampler with electron capture and nitrogen–phosphorus detectors. Quantification and confirmation analysis were performed using HP-5 (5% diphenyl–polysiloxane and 95% dimethyl–polysiloxane) and HP-1701 (14% cyanopropyl–phenyl methyl–polysiloxane) columns (30 m × 0.32 mm × 0.25-μm film thickness) for gas chromatography–mass spectrometry. The column was held initially at a temperature of 160°C for 1 min, then increased at 10°C min^{-1} to 200°C, and finally at 300°C for 15 min. Temperatures of the injector and detector were maintained at 250 and 325°C, respectively. Helium was used as a carrier gas at a flow rate of 2.0 ml min^{-1}. A linear relationship between concentration and peak area was obtained within the range of 0.5 to 2.0 ng μl^{-1} with correlation coefficients greater than 0.99. Method detection limits were greater than 1.0 $\mu g\,L^{-1}$ for OPs and 2.0 $\mu g\,L^{-1}$ for NMCs in water, and 0.02 and 0.04 $mg\,kg^{-1}$ in sediment, for OPs and NMCs, respectively.

Fish sampling and tissue preparation

A maximum of ten fish were collected from three fish species (*G. ceylonensis*, *D. malabaricus,* and *R. daniconius*) with cast-nets (10-mm stretched mesh size) and stored at –21°C until analysis. Only sexually mature fish were selected from mixed-sex populations in each site (Table 1). Gender neutrality [17,18,30,31] and seasonal stability [32,33] were assumed for basal AChE activity in fish species based on previous studies. Measurements were taken of fork-length (± 0.1 cm), total body weight (± 0.001 g), and brain weight (± 0.001 g) after thawing, and the eyes and dorsal muscle from the cephalic region were excised and weighed (± 0.001 g); all tissues were stored at –21°C until analysis. Thawed tissues were thoroughly homogenized in an Eppendorf homogenizer on ice with the help of a micropestle, and 1 ml of ice-cold 0.1 M phosphate buffer was added (pH 7.4; prepared by mixing mono- and dibasic sodium hydrogen phosphate; Sigma-Aldrich). Each tissue homogenate was adjusted to 20% volume (w/v) by adding appropriate volumes of 0.1 M phosphate buffer (pH 7.4). Then, 1 ml of the tissue homogenates was removed to Eppendorf tubes and centrifuged (Sanyo Micro Centaur) at 7,826 *g* for 5 min. The supernatant was used for AChE and protein analysis (see Supplemental References).

Acetylcholinesterase determination

Acetylcholinesterase activity was measured spectrophotometrically by using a modification of the Ellman et al. [34] assay as adapted to a microplate reader by Hemingway [35]. Acetylthiocholine iodide (Sigma-Aldrich) was used as the substrate, and the resulting thiocholine was reacted with the color-developing agent 5′-dithiobis-2-nitrobenzoic acid (Sigma-Aldrich), to give 2-nitro-5-thiobenzoic acid, a yellow-colored anionic product with maximum absorption at 405 nm. Briefly, two 25-μl replicates of the enzyme preparation were added to adjacent wells of a 96-well microtiter plate followed serially by 145 μl of 1% (v/v) Triton X-100 buffer (in 0.1 M of phosphate buffer, pH 7.8), 10 μl of 0.01 M 5′-dithiobis-2-nitrobenzoic acid (in 0.1 M phosphate buffer, pH 7.0), and 25 μl of 0.01 M acetylcholine iodide (in distilled water). Acetylcholinesterase activity was determined immediately for 5 min at 12-s intervals with a

Table 1. Descriptive data on fork-length (L) and total weight (W) of fish species used for AChE analysis[a]

Species	Site	No.	L ± SE	L (min)	L (max)	W ± SE	W (min)	W (max)	*p* value
GC	ME	20	13.36 ± 0.36	9.5	15.2	26.84 ± 2.40	12.24	46.15	0.126 (L)
	GI	19	12.58 ± 0.32	9.6	14.5	29.28 ± 1.96	12.89	43.26	<0.001 (W)
	ER	19	12.07 ± 0.35	9.8	15.5	26.14 ± 2.50	11.69	55.86	
	RO	10	12.09 ± 0.59	9.5	15.0	43.53 ± 6.79	12.38	77.20[b]	
	IK	10	11.01 ± 0.41	9.3	12.4	19.81 ± 2.05	10.97	27.85	
DM	ME	18	7.43 ± 0.16	6.4	8.5	5.86 ± 0.41	3.62	8.72	0.290 (L)
	GI	19	7.97 ± 0.31	6.5	10.9	7.22 ± 0.86	3.32	16.27	0.302 (W)
	RO	10	7.98 ± 0.17	7.2	8.7	6.88 ± 0.50	4.63	9.87	
	IK	10	7.92 ± 0.20	7.0	8.8	5.76 ± 0.41	4.12	8.24	
RD	ER	19	8.33 ± 0.21	6.7	10.2	8.19 ± 0.59	4.16	13.7	0.435 (L)
	RO	10	8.01 ± 0.26	6.5	9.7	6.40 ± 0.69	3.58	11.67	0.137 (W)
	RM	9	8.52 ± 0.29	7.4	9.6	8.25 ± 0.74	5.03	11.61	

[a] AChE = acetylcholinesterase; GC = *Garra ceylonensis*; DM = *Devario malabaricus*; RD = *Rasbora daniconius;* RO = Rambukoluwa; IK = Illukkumbura; RM = Ranamuregama; ME = Medawela; GI = Girambe; ER = Erabadda. RO, IK, and RM are reference sites in the Knuckles Conservation Forest area. Within a column, for each species, mean L and W among sampling sites are not significantly different (ANOVA, Tukey test $p > 0.05$).
[b] Significant difference from the rest of the sites for *G. ceylonensis*.

BioTek ELx808 microplate reader, and AChE specific activity was calculated by a computer-assisted program using the formula of AChE specific activity = optical density$_{405}$/ [P] × E, where OD_{405} is the optical density from AChE assay, P is the protein concentration in the well (mg ml^{-1}), and E is the extinction coefficient of the substrate (OD_{405} μmol^{-1} ml^{-1}). The AChE specific activity in fish tissues was given as μmol min^{-1} mg^{-1} protein.

All procedures were carried out on ice to minimize loss of enzyme activity. All assays were corrected for nonenzymatic activity with the same mixtures, except for using 25 μl of distilled water instead of the enzyme preparation. Results were expressed as a percentage of AChE activity in the inhibited fraction compared with control (uninhibited) activity. Protein concentrations of the individual homogenates were measured by Bio-Rad protein determination [36].

Statistical analysis

All data were first tested for normality and homogeneity of variance, and then analyzed by one-way analysis of variance (ANOVA), followed by a Tukey test using $p < 0.05$ as significant. Data are expressed as means ± SE. Acetylcholinesterase inhibition levels were calculated based on the mean AChE activity in control fish and are presented as a percentage (± SE). Calculations of basic descriptive statistics on primary data and statistical analyses were performed using Minitab, Version 16 for Windows.

RESULTS

Organophosphate and NMC contamination patterns

Only four (i.e., chlorpyriphos, diazinon, dimethoate, and profenophos) of five target OP insecticides and one (i.e., carbofuran) of three NMC insecticides were detected in any of the samples of subsurface water, runoff water, and surface sediment, irrespective of the season. In relation to seasonal effects, an increase in concentration of all the detected OP and NMC insecticides was found during the *Yala* season. The spatial variations of cumulative residue levels of OP and NMC insecticides in water and sediment matrices are shown in Figures 2 and 3, respectively. Water samples from background stream flow (A samples) and in field streamlets draining into the main flow (B sample) contained only chlorpyriphos (<1 μg L^{-1}), at some sites. On a few occasions, dimethoate, carbosulfan, profenophos, phenthoate, chlorpyriphos, and diazinon were detected between <1 μg L^{-1}, and 3 μg L^{-1}. A similar situation was also observed for stream runoff collected during two sampling efforts during the *Maha* rainfall events in October and December (2010): profenophos and chlorpyriphos were detected at 1 μg L^{-1} at some sites. Chlorpyriphos and diazinon were detected in sediments at concentrations of 16.36 mg kg^{-1} (dry wt) in the *Yala* season. The principle occurrence of other pesticides could not be determined because of concentrations less than the method detection limit.

Acetylcholinesterase activity in fish species

The mean (± SE) lengths of fish specimens were not significantly different (ANOVA, $p > 0.05$) between sites and were 12.0 (± 0.18, $n = 78$) (*G. ceylonensis*); 7.8 (± 0.13, $n = 57$) (*D. malabaricus*); and 8.3 (± 0.14, $n = 38$) (*R. daniconius*). However, a significant weight difference was found between *G. ceylonensis* individuals at some sites (Table 1).

Brain AChE activity was higher in January than July in *G. ceylonensis* at all three sites in the Uma-oya, whereas muscle and eye showed the same seasonal difference only at the Medawela site (Fig. 4). Brain activity levels were lower in July by 15.6% in Medawela; 26.3% in Girambe; and 36.4% in Erabadda than reference fish in the Knuckles (Table 2). The brain AChE activity in January samples from the Girambe and Erabadda sites were not significantly different compared with reference fish in the Knuckles. However, a significant inhibition of brain AChE activity of 29.5% was found in fish from the Medawela site in January compared with the Knuckles fish. All Uma-oya sites showed lower brain activity levels compared with the Knuckles reference populations (July; $n = 20$, $p < 0.001$) by variable inhibition degrees of 40.1 to 46.4%.

Significant differences ($p < 0.001$) were found between tissue-specific AChE activities and the specific activity decreased in sequence of brain > muscle > eye in *D. malabaricus*. In unexposed fish, specific activity of AChE in brain was 51% higher (twofold) than those in dorsal muscle (1.27 and 0.62 μmol min^{-1} mg^{-1} protein, respectively). Muscle AChE activity at all sites in all months in the Uma-oya was significantly depressed by 37.2 to 72.8% compared with the Knuckles

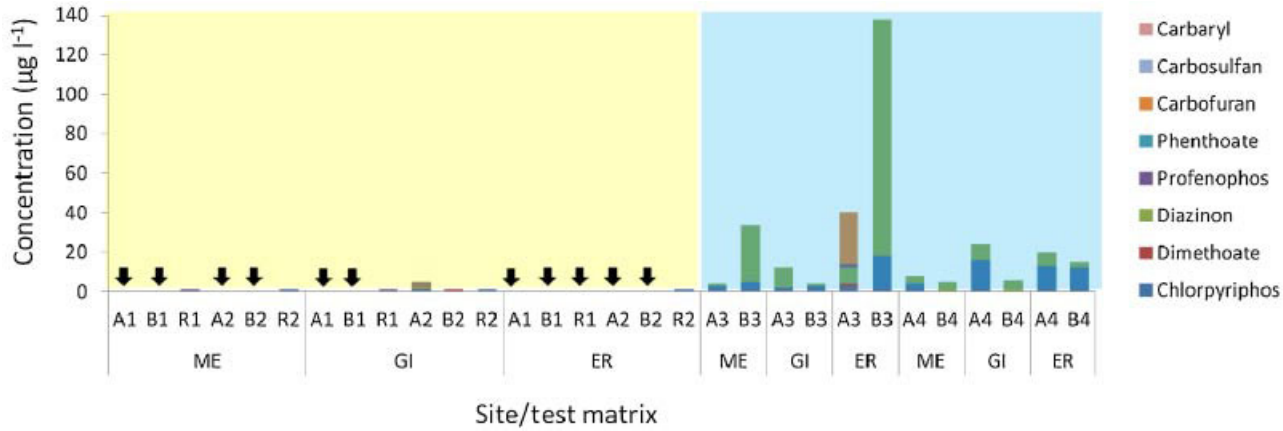

Fig. 2. Organophosphate (OP) and N-methyl carbamate (NMC) insecticide concentrations in the water samples collected in the Uma-oya catchment. Arrows denote sampling occasions on which pesticide concentrations were below the detection limit of the method (1.0–2.0 μg L^{-1}). Shaded areas indicate *Maha* (yellow area left) and *Yala* (blue area right) sampling seasons. Typically, the *Maha* season is October to January. Samplings were conducted in October (A1, B1, and R1) and December (A2, B2, and R2) in 2010. The typical *Yala* season is April to July. Samplings were conducted in June (A3 and B3) and August (A4 and B4) in 2011. A1 to A4 = mainstream subsurface water flow; B1 to B4 = field canal to main flow; R1, R2 = mainstream runoff; ME = Medawela; GI = Girambe; ER = Erabadda. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

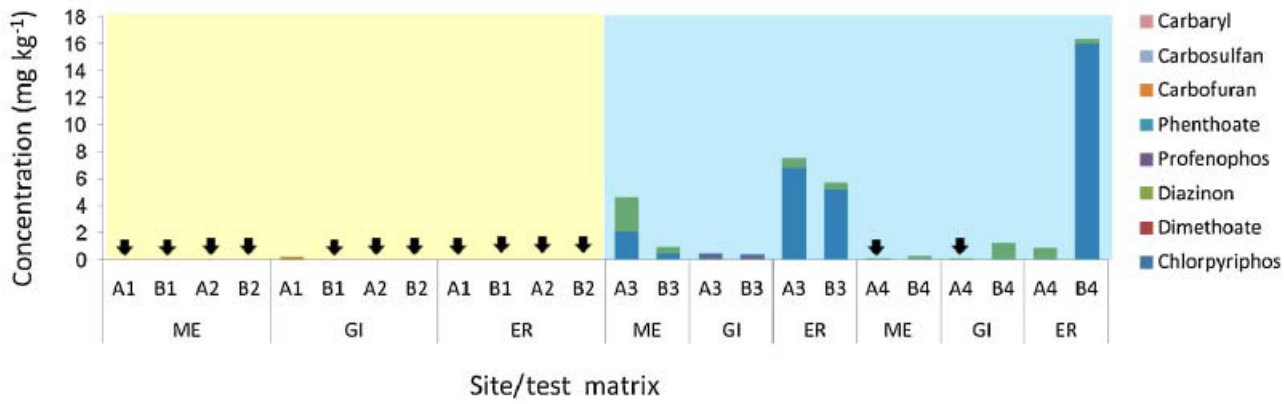

Fig. 3. Organophosphate (OP) and N-methyl carbamate (NMC) insecticide concentrations in the sediment samples collected in the Uma-oya catchment. Arrows denote sampling occasions on which pesticide concentrations were below the detection limit of the method (0.02–0.04 mg kg^{-1}). Shaded areas indicate *Maha* (yellow area, left) and *Yala* (blue area, right) sampling seasons. Typically, the *Maha* season is October to January. Samplings were conducted in October (A1 and B1) and December (A2 and B2) in 2010. The typical *Yala* season is April to July. Samplings were conducted in June (A3 and B3) and August (A4 and B4) in 2011. A1 to A4 = bed sediment in the mainstream flow; B1 to B4 = bed sediment in the field canal to main flow; ME = Medawela; GI = Girambe; ER = Erabadda. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

fish. In the Girambe site, muscle AChE activity was depressed by 44.8% between January ($n = 10$) and July ($n = 9$) in fish samples; seasonal AChE activity in the muscles was not significantly different in the Medawela and Erabadda sites (Fig. 4). The AChE activity in the eye at all sites in all seasons was significantly depressed ($p < 0.001$) by 42.8 to 75.9%, except at the Medawela site in January (2011), which showed a depression that was smaller by 17.9% (Table 2). No significant differences in AChE activities in eye tissues were found between January and July samples from the Uma-oya.

Rasbora daniconius was only available at Erabadda out of three sites in the Uma-oya. The AChE-specific activities in the control populations ($n = 17$) of *R. daniconius* tissues were observed in a sequence of decreasing sensitivity as muscle = brain > eye. The corresponding baseline AChE-specific activities (mean ± SE) were 0.76 ± 0.05, 0.67 ± 0.04, and 0.22 ± 0.02 μmol min^{-1} mg^{-1} protein, respectively. Although the specific AChE activity difference between the brain and the muscle was smaller (13%), and not significantly different (ANOVA, $p > 0.05$), eye activity was reduced by 67 to 71% ($p < 0.001$) compared with reference fish. No significant differences were found in the brain AChE in control fish and January fish from the Erabadda site (Fig. 5), although 60% inhibition was observed in July ($p < 0.0001$). A highly significant inhibition of 40 to 56% was found in muscle AChE activity in *R. daniconius* from the Erabadda site in January and July (2011) ($p < 0.0001$). Although not significantly different ($p > 0.05$), the muscle AChE activity was 27% less in July compared with January samples (Table 2). No differences were found in mean AChE activity in eye tissues from any of the populations in the Knuckles and Uma-oya sites ($p = 0.57$) (Fig. 5).

Devario malabaricus was available at two sites in the Uma-oya (Medawela and Girambe). The activity of AChE in the control population ($n = 10$) of *D. malabaricus* in the Knuckles streams was highest in muscle > brain > eye (Fig. 6). The dorsal muscle and brain AChE-specific activities in *D. malabaricus* were significantly different ($p < 0.001$) by 43% high (1.7-fold). Both brain and dorsal muscle specific activities had significant differences from the eye, which had the smallest activity (1.39, 0.80, and 0.24 μmol min^{-1} mg^{-1} protein, respectively). Except in the Girambe site in January, all other brain AChE activities were significantly different ($p < 0.001$) from the Knuckles

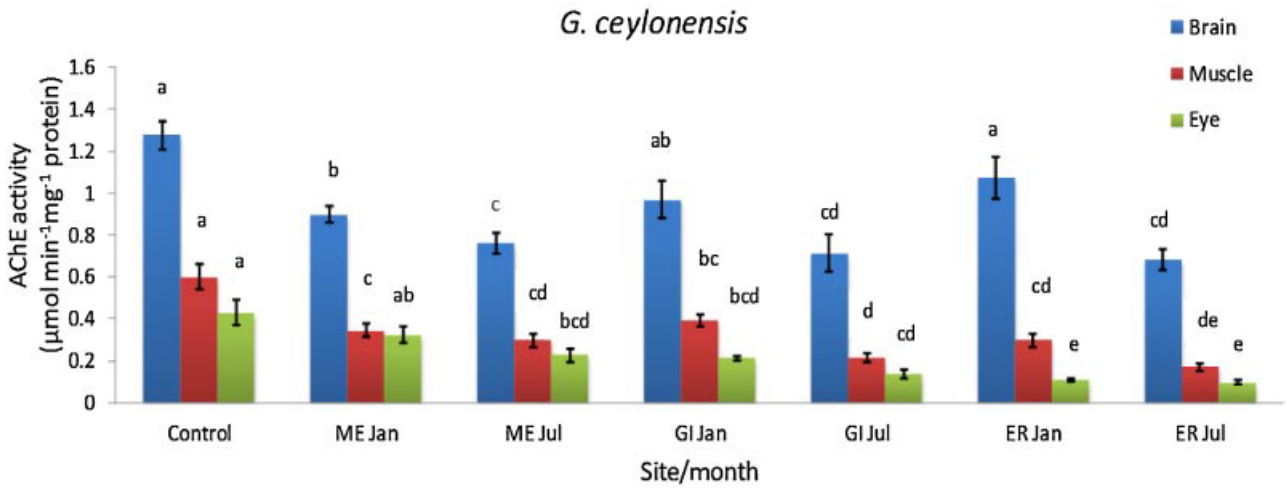

Fig. 4. Seasonal pattern of AChE activity in brain, muscle, and eye tissues of *Garra ceylonensis* in the Uma-oya compared with the Knuckles counterparts. Values are mean ± SE. Means of each tissue that do not share a letter are significantly different (ANOVA, Tukey $p < 0.05$) compared with control. ME = Medawela; GI = Girambe; ER = Erabadda. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

Table 2. In vivo inhibition of AChE activities in brain, muscle, and eye homogenates from *G. ceylonensis*, *D. malabaricus*, and *R. daniconius* in relation to the background cumulative exposure concentrations of OP and NMC insecticides in the Uma-oya in the *Maha* (2010) *Yala* (2011) seasons

Site	Season	Cumulative residue concentration ($\mu g\,L^{-1}$)[a]	Fish species	Brain (% inhibition)[b]	Muscle (% inhibition)[b]	Eye (% inhibition)[b]
ME	*Yala*	38	GC	40.1	58.3	42.8
			DM	41.8	59.3	27.5
	Maha	ND[c]	GC	29.5	44.6	17.9
			DM	34.2	54.1	NIL
GI	*Yala*	46	GC	43.9	65.4	65.8
			DM	47.8	64.6	7.7
	Maha	6	GC	23.9	37.2	46.4
			DM	3.7	28.2	NIL
ER	*Yala*	213	GC	46.4	72.8	75.9
			RD	59.9	56.1	15.9
	Maha	ND[c]	GC	15.8	52.5	72.5
			RD	4.4	39.9	NIL

[a] Total OP and NMC insecticides measured in the water samples.
[b] Each value represents the percentage of acetylcholinesterase (AChE) activity in the inhibited fraction compared with the control (uninhibited) activity.
[c] Pesticide concentrations were below the method detection limit, 1.0–2.0 $\mu g\,L^{-1}$.
GC = *Garra ceylonensis*; DM = *Devario malabaricus*; RD = *Rasbora daniconius*; ME = Medawela; GI = Girambe; ER = Erabadda; ND = not detected; NIL = no inhibitory levels.

individuals. The minimum and maximum inhibitions on brain AChE between the Knuckles and the Uma-oya populations were within a range of 34.2 to 47.8%, respectively. No significant differences were found in brain AChE activity (11.5%) between January and July samples in the Medawela site, whereas it was inhibited by 45.9% ($p < 0.001$) between January and July samples in the Girambe site.

The muscle AChE activity of all fish in the Medawela and Girambe sites in the Uma-oya in both seasons were significantly depressed by 28.2 to 64.6% compared with the Knuckles counterparts. A depression in muscle AChE activity was found between January and July in the Medawela (11.4%) and Girambe sites (50.7%); only the latter was highly significant ($p < 0.001$). No significant differences ($p > 0.05$) were seen in mean AChE activity in the eye in any of the populations in the Uma-oya sites compared with the Knuckles (Fig. 6).

DISCUSSION

Contamination pattern

Organophosphate and NMC pesticides were detected at all sites in water during the *Yala* season, and rarely and only in low concentrations during the *Maha* monsoon season. The *Yala* vegetable cultivation season (June–September) is relatively drier than the *Maha* season in the Uma-oya catchment, and pesticides are used more heavily during the cultivation of vegetables compared with rice. The small-scale vegetable fields are exorbitantly fertilized [37], and large amounts of pesticides (>23 kg of active ingredients ha^{-1} $year^{-1}$) are used for pest and disease control [21]. Chlorpyriphos, diazinon and carbosulfan had the greatest amount of agricultural application in the catchment [25] and chlorpyriphos, diazinon, and carbofuran were the dominant pollutants found. Chlorpyriphos and diazinon were detected in sediments at concentrations of 16.36 $mg\,kg^{-1}$ (dry wt). The Uma-oya catchment is characterized by exceptionally high soil erosion associated with topography (i.e., sloping lands), cropping patterns, and high intensity monsoonal rains. The entry and occurrence of pesticides in the Uma-oya catchment has not been studied, but similar catchment studies in Sri Lanka [8] and elsewhere [38] have shown that contamination of the mainstream originates from adjacent fields and inputs into tributaries mainly via surface runoff and to a lesser extent by spray drift.

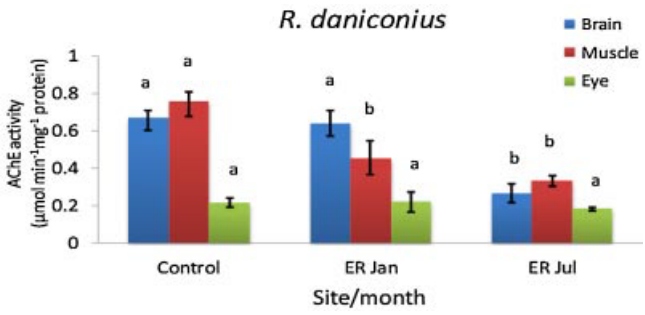

Fig. 5. Seasonal pattern of AChE activity in brain, muscle, and eye tissues of *Rasbora daniconius* in the Uma-oya in comparison with the Knuckles counterparts. Values are mean ± SE. Means of each tissue that do not share a letter are significantly different (ANOVA, Tukey $p < 0.05$) compared with control. ER = Erabadda. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

Despite the shorter lifespan of OP and NMC pesticides in environmental matrices, concentrations up to 138 $\mu g\,L^{-1}$of OPs were detected in the present study (total of chlorpyriphos and diazinon). Menike et al. [39] reported rainfall-induced chlorpyriphos concentrations of 2.48 $\mu g\,L^{-1}$ in a year-round assessment of stream water irrespective of doses applied in a similar agricultural catchment. Vegetable cultivation in the *Yala* season depends principally on nonrain–fed irrigation methods; the crops are irrigated with water taken from irrigation canals usually at 2- to 3-d intervals, and the tail water can be tainted with pesticides that have been applied during the preceding days or weeks. The upper and lower catenae of the lowland fields are composed of sandy–clay–loam to sandy–loam agricultural soils (with low organic matter content, 1.0–2.3%), affecting soluble and adsorbed fractions of pesticides in receiving waters.

Acetylcholinesterase activities

Higher basal values of muscle AChE activities in *D. malabaricus* and *R. daniconius* were observed in reference fish, whereas *G. ceylonensis* had higher basal activity in the brain. Lower basal activity of AChE was observed in the eye tissue in all species tested. Acetylcholinesterase activity in various fish tissues (e.g., brain, muscle, liver, eyes) has been used to indicate exposure to OP and NMC pesticides [6,16]. The brain AChE activities measured in *R. daniconius* in the reference area are in the same order of magnitude as those reported by Wijeyaratne

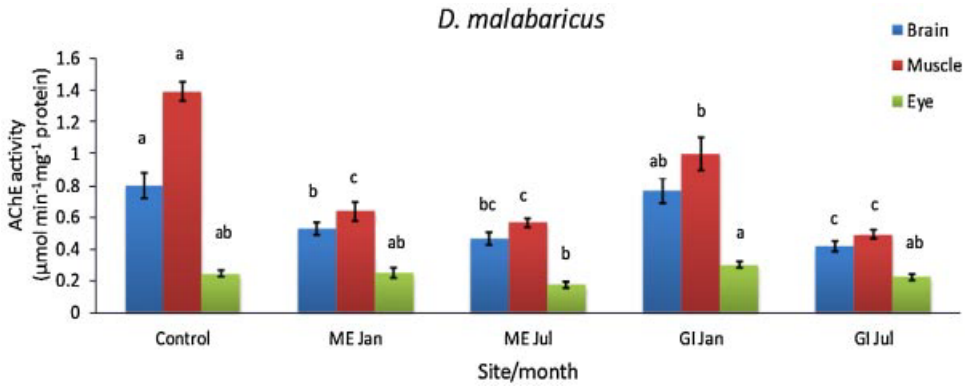

Fig. 6. Seasonal pattern of AChE activity in brain, muscle, and eye tissues of *Devario malabaricus* in the Uma-oya in comparison with the Knuckles counterparts. Values are mean ± SE. Means of each tissue that do not share a letter are significantly different (ANOVA, Tukey $p < 0.05$) compared with control. ME = Medawela; GI = Girambe. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

and Pathiratne [9] from *R. caverii* (a congeneric species) in a rice-growing area in the wet zone of Sri Lanka, although these authors reported AChE activity per whole body weight.

It has been suggested that a correlation exists between the general physical activity of fishes and their levels of skeletal muscle AChE, with higher AChE activities present in active than sluggish species [40]. *Rasbora daniconius* and *D. malabaricus* showed higher levels of muscle AChE, and both species are active swimmers in the water column [41]. In contrast, the pronounced brain AChE activity in *G. ceylonensis* compared with muscle is in accordance with its relatively sedentary nature as a benthic fish adapted for living on stony stream bottoms [41].

Acetylcholinesterase inhibition

Acetylcholinesterase inhibition in fish brain did not exceed 75% in any of the sites in any of the fish species; the highest inhibitions were in the range of 34.2 to 60%. A 73% inhibition in muscle AChE activity in *G. ceylonensis* was associated with intense exposure months in the *Yala* season. More interestingly, AChE inhibition was greater than 70% in *G. ceylonensis* eyes in both *Yala* (76%) and *Maha* (72.5%) seasons, indicating particular sensitivity of eye tissue to inhibitors.

The highest inhibition of AChE (up to 60% in brain and up to 56% in muscle AChE activity in *R. daniconius)* occurred during the low-flow month in July (*Yala* season), when pesticide residues were more frequently detected. In lake environments (where pollutant accumulation and storage can be more likely), muscle ChE activities in Nile tilapia were inhibited by 37 to 46% during the rainy periods in comparison with the low extent of inhibition (21–25%) during the dry periods [10]. At the downstream site (Girambe), fish species showed more inhibition of AChE activity (both brain and muscle) than at the upstream (Medawela) site in the mainstream of the Uma-oya. Galgani et al. [30] demonstrated that AChE activity in the muscles of the North Sea dab (*Limanda limanda*) varied according to the contamination gradient and was higher in less polluted waters.

Inhibited AChE activity in the muscle was apparent in *D. malabaricus* without significant responses in brain activity under low exposure periods to OPs and NMCs. Muscle AChE activity is thought to provide a protective role against the brain AChE inhibition in mosquito fish by supplanting active inhibitors (e.g., chlorpyriphos) [42]. Acetylcholinesterase inhibition in eye tissue was less dramatic in *D. malabaricus* and *R. daniconius*.

The pattern of AChE inhibition is apparently stronger in different tissues of *G. ceylonensis* living in the tributary site (Erabadda) than in mainstream sites (Medawela and Girambe) in the Uma-oya. The high AChE activity in "clean" environments and reduced activity in "polluted" areas is fairly typical [30,32]. However, more detectable pesticide residues were found in the tributary site than in mainstream sites, with no contrasting differences in cropping and pesticide use patterns in the three study sites [39]. Despite high pesticide inputs into the stream through associated field canals (as has been observed at the Erabadda site), the differences in total OP and NMC concentrations in two mainstream locations (4–24 $\mu g\,L^{-1}$) and the tributary location (20–40 $\mu g\,L^{-1}$) were not associated with different AChE inhibition. This is not surprising because similar AChE inhibition (86–92%) has been seen in fish brain under diazinon exposures over a range of 42 to 450 $\mu g\,L^{-1}$ during 24- to 96-h exposure [43,44] (see Supplemental References).

Although AChE activity inhibition has been correlated with dominant AChE-inhibitory compounds, the narrow range of SE values (0.01–0.10) for mean-specific AChE activity (data not shown) suggests a wider exposure to contaminants (i.e., diffuse nature of contaminants), as variability can be higher with localized point pollution [31,32]. Other AChE-modulating compounds were present that were not detected frequently. For example, carbofuran, a strong AChE inhibitor, was detected in one of the three sites in the Uma-oya (i.e., a tributary drainage at Erabadda) at a concentration of 16 $\mu g\,L^{-1}$, which has the potential to inhibit fish brain AChE up to 59 to 80% at 10 to 50 $\mu g\,L^{-1}$ [45,46] or even lower concentrations [47].

Inhibition of AChE activity has mainly been reported in studies performed with pesticides [18], but different effects have also been seen with metals on fish brain [48] and muscle AChE activity [48], including cadmium [49], mercury, and lead [50]. Miron et al. [51] reported increased AChE activity in fish tissues by some contemporary classes of herbicides such as quinclorac and metsulfuron–methyl. Therefore, AChE inhibition as an effective biomarker of pesticides in real-exposure situations, such as in a diffused agricultural exposure scenario, is rather limited [11] unless it is known that no other anticholinesterase agents are present in the system at concentrations that may cause AChE inhibition. Earlier studies in the Uma-oya

stream and tributary streams reported excess Pb and Cd concentrations in water and sediment close to agricultural and urban areas [52,53] (see Supplemental References).

Wijeyaratne and Pathiratne [9] showed *R. caverii* to be markedly more sensitive to brain AChE depression compared with exotic tilapia (*O. mossambicus*), suggesting that *Rasbora* may be more sensitive than exotic species. Many organisms produce different cholinesterasic forms in their muscle tissue [54], and tilapia have multiple forms [55], but cyprinid fishes have shown AChE activity only in their muscles [56]. *Danio rerio*, which is one of the closely related lineages to *D. malabaricus*, is confirmed to have only one functional AChE gene [12]. Thus the heightened tolerance of exotic tilapian species [6] may be due to their diverse forms of cholinesterase activities.

The less dramatic AChE inhibition in the eye tissues in *D. malabaricus* and *R. daniconius* in both seasons indicates another example of the protective capacity of muscle AChE in fish. The existence of no AChE inhibition in the eye tissues despite mild (39.9% in the Erabadda site) or higher (54.1% in the Medawela site) inhibitory effects on muscle AChE activity is consistent with the above observation. Many planktivorous fish are visual foragers, highly dependent on light to efficiently detect and consume their zooplankton prey. Therefore, it can be argued that the protective capacity of muscle AChE for the brain (as discussed above) can be extended to other tissues that have critical importance for survival. The visually aided feeding habit of *R. daniconius* has been confirmed by Pet and Piet [57] by their preference to concentrate on the surface and in littoral zones in open waters. However, *G. ceylonensis* behaved dissimilarly; this fish species is a bottom-dwelling algae-eater (benthivorous), for which intense visual activity may not be advantageous. Therefore, it can be suggested that inherently low "buffering capacity" of fish tissues can be exploited as sensitive AChE assessment targets in environmental monitoring.

Possible implications for fish survival

The range of AChE inhibitions seen was generally $<60\%$. A 20% or greater inhibition has been shown to indicate a mild exposure situation [58], and life-threatening exposures are thought to occur at greater than 70% [6]. Fulton and Key [6] reported instances of survivability of some fish species under intense inhibition in excess of 95% in brain AChE. Chandrasekara and Pathiratne [46] reported that the maximum inhibition of AChE in brain tissues at which mortality of *O. niloticus* occurred was 85% for chlorpyriphos (i.e., an OP insecticide) and 64% for carbosulfan (i.e., a NMC insecticide). Several studies have shown less correlation between muscle cholinesterase activity and swimming performance and/or velocity in fish exposed to fenitrothion (an OP insecticide) and carbofuran (a NMC insecticide) [59].

The severity of inhibition can depend on the compound and the fish species. Carr et al. [42] showed that brain AChE was inhibited up to 87 to 93% in largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and golden shiners (*Notemigonus crysoleucas*), whereas only 73% inhibition was found in the mosquitofish (*Gambusia affinis*), with no lethality after exposure to the same concentrations of chlorpyriphos. Recently, a whole-effluent environmental risk assessment approach in agricultural catchments has highlighted the delineation of complex-mixture ecotoxicological hazards to aquatic organisms [60]. Our recent investigations [61] revealed that in sites receiving high agricultural inputs (i.e., the middle catchment of the Uma-oya), suboptimum gonadal development and fecundity were present in Ceylon stone sucker (*G. ceylonensis*) populations, suggesting a gross impairment of fish physiology. This particular effect-based assessment approach is based on the premise that holistic adverse effects of the water bodies expressed as "effect" can be observed at lower levels of biological organization [62]. One key challenge to understanding this relationship is linking pesticide effects in individual fish to the intrinsic productivity of populations.

It has been shown that AChE inhibition predisposes fish to a number of physiological processes. Sandahl et al. [58] began to address this relationship by showing that exposures to low, environmentally realistic concentrations of OPs (e.g., chlorpyriphos) have implications on several bioenergetic functions. Acetylcholinesterase inhibition in fish can result in loss of equilibrium, decreased respiratory effectiveness, altered swimming performance, and reduced food consumption [13,40], the most commonly reported effects of OP and NMC exposure. The reductions in feeding are likely to lead to reductions in the size of gonads and fecundity, an end point that has been shown to be an important determinant of ecological competence and survival in individual fish [63]. Dutta and Maxwell [63] showed that a sublethal dose of $60\,\mu g\,L^{-1}$ of diazinon changed the microscopic structure of ovaries in bluegill (*Lepomis macrochirus*) including destruction of follicles, increased intrafollicular spaces, vacuolated cytoplasm, increase of atretic follicles, and shrinkage, leading to the production of fewer viable eggs and affecting the population dynamics of species in polluted environments.

Up to 85% inhibition of muscle AChE activity has been observed in *O. niloticus* during a 96-h exposure to sublethal doses of trichlorfon (an OP insecticide) [64]. The toxic effects from continuous exposure (i.e., in vitro experiments) are not always adaptable to intermittent exposure events occurring in the field [65]. At present, findings are conflicting as to whether the condition factor of fish species would substantiate pesticide impact under in situ conditions. In previous field studies in streams adjacent to rice fields, Wijeyaratne and Pathiratne [9] observed that the condition factor of *R. caverii* could not be interpreted as effects of pesticides and concurrent AChE inhibition.

An intense inhibition of AChE activity in the eyes of *G. ceylonensis* up to 76% can be potentially severe enough to disrupt the overall survivability of fish in their natural environments, if not life-threatening. In polluted environments, the suppression of eye AChE activity in fish might lead to impaired optomotor response (i.e., the ocular reaction to movement or light resulting in body motion either toward or away from the stimulus). Pan and Dutta [44] observed limited optomotor reactions in largemouth bass, *Micropterus salmoides*, that is, disrupted food-searching, orientation toward food odor, searching for mates, and locating and avoiding predators after exposure to sublethal concentrations of diazinon. Sahib et al. [66] stated that differential inhibition of AChE activity in fish tissues (i.e., brain, muscle, eyes) may be due to the presence of isozymes with different affinities for the substrate and the inhibitor. They also stated that the inhibitory pesticides can be present in different amounts in the different tissues, producing differential inhibition, or the inhibitor may be metabolized at different rates.

In conclusion, evidence from the present study confirms that AChE inhibition is useful as an exposure biomarker for the presence of neurotoxic contaminants in the agricultural catchments of Sri Lanka. Exposure to AChE-inhibitory OP and NMC

insecticides is shown to be higher in the *Yala* season than in the *Maha* season in the Uma-oya catchment. Inhibition in fish species showed the same concentration pattern of AChE-inhibitory OP and NMC insecticides across seasons. In the present study, because control fish samples were caught in the *Yala* season, and no significant differences were found in stream temperature, air temperature, and rainfall profiles between catchments [21], the seasonal influence on baseline AChE activity was ignored. However, divergent opinion in the literature exists as to whether temperature influences AChE activity in fish [32,33,67], thus preventing extensive generalizations. Even though it is impossible to correlate the findings of AChE inhibition with the presence of exclusive OP and NMC insecticides, the results indicate that other contaminants can act synergistically, cumulatively, or antagonistically in complex mixtures contributing to the overall impact on AChE activity. However, determining AChE activity and using this biomarker as an early warning signal of exposure and/or adverse effects can still be effective in the future to study spatial and temporal trends in the quality of waters running in agricultural catchments.

Our findings, along with previous observations of soil transport into the stream, have implications for planning irrigation and soil conservation measures in the Uma-oya catchment in terms of aquatic life conservation. If contaminant discharge is a serious problem during the *Yala* cultivation season, management options could focus on specific issues occurring in the system. Depending on the stream section where active land preparation practices prevail, flashy currents of soil-laden water could also occur, and these would transport pesticides off the vegetable fields. Cristen et al. [68] noted some regulatory options instituted in Australia to curtail pesticide movement from the farm field, that is, by practicing a withholding period after pesticide application during which water is not released from the fields and constructing high banks to ensure rainfall is retained within the field. However, this is not practically feasible in a location like the Uma-oya catchment. This is primarily a question of farm field size and soil/geological characteristics. Stringent pesticide management options and good agricultural practices would be required to protect fish species in agricultural catchments.

SUPPLEMENTAL DATA

Supplemental References 1-20 (90 KB PDF).

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Review

Assessment of Health Risk in Human Populations Due to Chlorpyrifos

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Abstract: A wide ranging survey was carried out of the available data from ten different countries on human exposure to chlorpyrifos, in many different occupational and nonoccupational settings. Low levels of chlorpyrifos residues were found to be widely distributed in the global human population, but most of these do not constitute a public health risk, as evaluated using the U.S. Environmental Protection Agency (USEPA) Guidelines. For example, the general populations in USA, Germany and Italy had detectable residue levels well below the guidelines. However, high levels of health risk were apparent in a specific group of pregnant mothers in the USA, at median exposure with a $HQ_{0.50}$ of 26.6, suggesting that most of this population group was affected. Also the high exposure group (5% most exposed) with occupationally exposed manufacturing workers in the USA had a $HQ_{0.95}$ of 2.6 to 42.0, and pest control applicators in Australia and the USA both had a $HQ_{0.95}$ of 5.2. Some farmers in Sri Lanka and Vietnam had a high level of risk after spraying applications, having a $HQ_{0.95}$ of 2.2 and 19.5 respectively at the high exposure level. These results suggest that there is a possibility of adverse health effects in specific population groups in many different settings throughout the world.

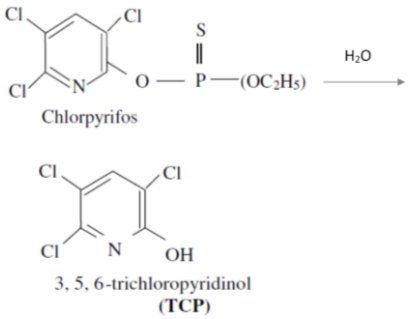
Keywords: farmers; pest control applicators; manufacturing workers; hazard quotient; USEPA guidelines; chronic population dose; post application dose

1. Introduction

The organophosphate insecticide, chlorpyrifos, has broad spectrum activity against many arthropod species. It was introduced to the market in 1965 and now plays a major role in controlling a range of pests in agricultural crops (rice, vegetables and fruit crops) and has a variety of other uses in pest control [1]. There are several manufacturers throughout the world and it is common in many countries. It is an acetylcholinesterase inhibiter and considered to incur potential adverse effects as a result of occupational exposure [2].

Chlorpyrifos has a relatively nonpolar molecule (see Figure 1) with low aqueous solubility (2 mg/L), high log K_{ow} (6) and relatively low persistence in the environment. The major metabolite in biological systems is 3,5,6-trichloro-2 pyridinol (TCP) which is passed in the urine of mammals as shown in Figure 1. Its insecticide properties (or its metabolites) are related to its ability to inhibit cholinesterase (AChE) [3] which affects nervous function and leads to severe and often lethal biological damage in organisms [4]. In humans, chlorpyrifos can inhibit the AChE enzyme in the central and peripheral nervous systems, causing adverse effects within hours of exposure [5,6]. Inhibition of plasma cholinesterase damages the central, sympathetic and parasympathetic nervous systems due to its inactivation at the sites of white matter in CNS, pancreas and heart [5]. Guidelines to protect human health have been recommended by many agencies, for example the U.S. Environmental Protection Agency (USEPA) [1].

Figure 1. Chlorpyrifos and its hydrolysis product, trichloropyrridinol (TCP).



Many instances of occupational exposure to pesticides, including chlorpyrifos, have been recorded [2,7–11] and application of pesticides to fruit and vegetables can also contribute to pesticide exposure through the diet. Use of pesticides can result in residue levels in commodities and in the immediate environments, such as soil, biota and aquatic systems. An extensive data base is now available on human exposure in the scientific literature.

In previous work we have evaluated the health risk due to the use of chlorpyrifos by rice farmers in Vietnam [12]. The exposure levels were found to exceed the acute exposure guidelines of various countries [13]. In addition, the risk was characterised using various probabilistic techniques indicating

a health risk [14]. Also we have developed additional methods for the characterisation of health risk using probabilistic distributions [15].

An extensive evaluation of the ecological risk of chlorpyrifos to aquatic environments in North America has been carried out [16]. On the other hand, few human health risk assessments [2] on chronic exposure to low doses of chlorpyrifos have been conducted. Although many exposure evaluations are available, there are no studies that have evaluated the human health risk as a result of dietary exposure to chlorpyrifos. There is therefore a need for an evaluation of the existing data on chlorpyrifos exposure, resulting from occupational and nonoccupational usage, to assess the risk to human health on an international basis.

The aim of this study was to assess the level of risk to human health resulting from exposure to chlorpyrifos with international populations by comparing reported exposure data with established criteria to establish the health risk.

2. Methodology

2.1. Strategy Used for Risk Assessment

The exposure assessment for the human populations in many countries was carried out with reported data from the scientific literature on the occurrence of the chlorpyrifos metabolite and biomarker, 3,5,6-trichloro-2 pyridinol (TCP), in urine (see Figure 1). This data was used to calculate the exposure to chlorpyrifos as the Equivalent Chlorpyrifos Ingested Dose (ECID). This data was plotted as Cumulative Probability Distributions (CPD) with Cumulative Probability plotted *versus* log ECID. This allowed the segmentation of the exposed population into low exposure group (at the 0.05 cumulative probability exposure level); the median exposure group (0.50 cumulative probability exposure level); and the high exposure group (at the 0.95 cumulative probability level).

Guideline Values for chlorpyrifos have been established by various agencies and are available as measures of the threshold dose for adverse effects (see Table 1). The most comprehensive of these are those developed by the US EPA so it was decided to use these for this assessment. This allowed the calculation of the Hazard Quotient (HQ) values [HQ = Exposure/Guideline Value (GV)] to characterise the health risk. In terms of the exposure group being evaluated the Hazard Quotient for the low exposure group was described as $HQ_{0.05}$, the median group as $HQ_{0.50}$ and the high exposure group $HQ_{0.95}$. But the low exposure group was not evaluated in any population since the other higher exposure groups were considered to represent a conservative evaluation of the health risk in any group.

Units: It was decided to use the same units throughout this paper and the most applicable was ng/kg/day (nanograms/kilogram body weight/day).

2.2. Sources of Exposure Data and Calculation of the Equivalent Chlorpyrifos Ingested Dose (ECID)

2.2.1. Background

A literature survey was carried out on reported data on chlorpyrifos exposure in human populations throughout the world which was available as the TCP levels in urine. The data on the 3,5,6-trichloro-2-pyridinol (TCP) levels in human urine in different populations was used to estimate the Equivalent

Chlorpyrifos Ingested Dose (ECID). Eaton *et al.* [17] point out that with nonoccupationally exposed populations there may be errors due to the possible occurrence of TCP in the urine due to its presence in food and other sources. However this comment is not applicable to acute exposure in an application event where TCP is measured both before and after the event as described below. This comment requires a detailed evaluation of the occurrence of chlorpyrifos and TCP in food and how their toxicokinetics are affected by dietary and physiological factors. TCP is also one of the main metabolites of chlorpyrifos methyl which is used as an insecticide in agriculture with annual usage in the USA of less than 1% of chlorpyrifos [18]. Therefore it can be assumed that chlorpyrifos methyl is unlikely to be a source of significant exposure in the general population. Also the toxicology and public health effects of exposure to TCP from food and water have not been subject to thorough evaluation. Similarly chlorpyrifos oxon may play a role in the exhibited adverse effects of chlorpyrifos [19]. However it is noteworthy that Price *et al.* [20] have found that a "source to outcome" model relating dietry exposure to chlorpyrifos, as measured by TCP, to health outcomes as reasonably consistent with published results.

The TCP is usually reported in units of μg/L urine or as μg/g creatinine. When measured in units of mass/volume in the urine it is subjected to the variation of daily volume of urine eliminated by the person which can vary with hydration status. However the daily mass of creatinine excreted by a person is considered to be approximately constant [18,21]. Therefore it is assumed that the concentration, with creatinine correction, is a more reliable measurement, despite of the variations with different age groups, ethnicities *etc.* [21,22].

2.2.2. Exposure from Chronic Nonoccupational Activities

The ingestion of contaminated food & water, dermal contact with contaminated soil and plants and inhalation of contaminated air, are the possible pathways giving the baseline exposure to chlorpyrifos. Based on the half life of excretion of TCP in humans [23] it is assumed that a continuous daily exposure to chlorpyrifos results in a steady state for the TCP levels in urine [4,22]. However it has been suggested by Eaton *et al.* [17] that levels of TCP present in the food and other sources can lead to estimations of higher concentrations values for chlorpyrifos than actually occur. In this current investigation it is assumed that spontaneous, or *spot*, urine samples reflect the exposure within the previous 3 to 5 days [23]. An evaluation by Attfield *et al.* [24] into the use of *spot* sampling indicates that it requires the use of several sample measurements to achieve an accurate evaluation. This approach is supported by the outcomes of our previous research [12,13]. Results from these samples can be compared to the Guideline Values for chronic exposure in Table 1, eg CRfD. The method used to estimate the daily dose developed by Garabrant *et al.* [4] was modified to convert TCP levels (ng/g creatinine) in urine into Equivalent Chlorpyrifos Ingested Dose (ECID in ng/kg body weight/day) as expressed by the following equation

$$ECID = 1.4TCP\ (CPF_{MW}/TCP_{MW})\ CR/BW \quad (1)$$

where CPF_{MW} and TCP_{MW} are the molecular weights of chlorpyrifos (350.6 g/mole) and 3,5,6 trichloro-2 pyridinol (198.4 g/mole) respectively; CR, the mass of creatinine excreted per day (g/day); BW, the body weight of the subject (kg) and 1.4, a factor to correct for the total amount ingested

considering 70% partial absorption of the oral intake [23]. An average body weight of 70 kg was used unless otherwise specified. In general the average adult was considered to have a daily average urine volume of 1.7 L/day with creatinine at a mean concentration of 1.3 g/L [18,25]. Thus the Daily Average Adult Creatinine Excretion (DAACE) is expressed as

$$\text{DAACE} = 1.3 \text{ g/L} \times 1.7 \text{ L/day} = 2.2 \text{ g/day} \approx 2 \text{ g/day} \quad (2)$$

Considering the potential low hydration status in the field working environment the average elimination rate of creatinine was considered as (1.3 g/L × 1 L/day = 1.3 g/day) ≈ 1 g/day with the farmers, pest control applicators and manufacturing workers.

2.2.3. Acute Exposure from an Application Event

Evaluation of acute exposure after a chlorpyrifos spraying event was carried out using a modified procedure [26] and using the acute exposure guidelines in Table 1, e.g., ARfD. Urine samples were provided before an event (pre-application) representing the baseline exposure, as well as five daily samples after the event (post-application) representing the exposure due to the event. The samples were analysed for TCP as outlined above with the post-application levels corrected for the baseline (pre-application) and thus they represent the exposure due to the event alone. It is noteworthy that the comment made by Eaton et al [17], mentioned above, regarding occurrence of TCP in the diet and other sources causing over estimation of chlorpyrifos levels is not applicable with this procedure. This is due to the estimation of TCP being corrected for the occurrence of TCP pre-event. The TCP representing overall acute exposure is obtained by summation of the values obtained from the five days post-exposure urine samples [26].

2.3. Guideline Values (GV) Developed by Various Regulatory Agencies

Guideline Values have been established by the U.S. Environmental Protection Agency (USEPA) [1], the World Health Organization (WHO) [27], the Australian Department of Health and Aging (ADHA) [28] and several other agencies representing the critical levels for exposure to chlorpyrifos as in Table 1. The USEPA guidelines are currently under review and may change when finalized [29]. Since these values are expressed in the same units and based on oral intakes, they are comparable with the Equivalent Chlorpyrifos Ingested Doses (ECID) calculated as described above. Generally these Guideline Values (GV) are derived from the No Observed Adverse Effect Levels (NOAEL) of plasma or red blood cells cholinesterase (ChE) inhibition with surrogate animal species (rats, dogs and mice) and humans. The NOAEL is divided by a Safety Factor (SF) or Uncertainty Factor (UF) to establish the Guideline Value. The exceedance of the guideline values set by this procedure represents a health hazard but the specific nature of this hazard is not defined. It should be noted that with the CPAD (Chronic Population Adjusted Dose), the guideline for exposure of children and females from 13 to 50 years of age, no additional biological test data was used but an additional safety factor of 10 was used. Since the USEPA values (see Table 1) are the most comprehensive only these Guideline Values were used in this evaluation. The term *general* is used in this Table to describe chronic exposure in the general population.

Table 1. Examples of guideline values (GV) developed by various agencies.

Guideline description (applicable population group)	Agency	Dose (log dose) (ng/kg/day)	Reference
ARfD [a] (acute exposure group)	USEPA	5.0×10^3 (3.7)	[1] USEPA, 2000
APAD [b] (acute exposure children and females 13–50 years)		0.5×10^3 (2.7)	
CRfD [c] (general)	USEPA	0.3×10^3 (2.5)	
CPAD [d] (children and females 13–50 years)	USEPA	0.03×10^3 (1.5)	
ADI [e] (general)	WHO	10.0×10^3 (4.0)	[27] JMPR, 1999
ADI [e] (general)	ADHA	3.0×10^3 (3.5)	[28] Australian Government, 2008

[a] ARfD—Acute Reference Dose; [b] APAD—Acute Population Adjusted Dose; [c] CRfD—Chronic Reference Dose; [d] CPAD—Chronic Population Adjusted Dose; [e] ADI—Acceptable Daily Intake.

3. Occurrence of Chlorpyrifos in International Populations

3.1. Equivalent Chlorpyrifos Ingested Dose (ECID) in Populations in the USA

3.1.1. Individual Farmers

Scher *et al.* [26] reported equivalent chlorpyrifos mass (μg), taken up during a spraying event with twelve farmers in South Carolina and Minnesota. The participants were randomly selected during 2000 and 2001 from licensed pesticide applicators, recruited in a survey known as the Farm Family Exposure Study. The total body absorbed dose (ng) in exposed individual farmers was calculated using TCP levels corrected for the baseline exposure. This data was used in this current study to estimate the ECID, by dividing the mass with an average body weight of an adult as shown in Equation (3). The exposure was assumed to be continuous on a daily basis.

$$\text{ECID} = \text{CEM}/(\text{day} \times \text{BW}) \quad (3)$$

where ECID is in ng/kg body weight/day and CEM, the Chlorpyrifos Equivalent Mass in ng.

The data are presented in Figure 2A with the ARfD and CRfD values for comparison (see Table 1). The ECID levels were distributed between 400 and 7300 ng/kg/day (2.6 and 3.9 log scale) with a slope of 0.7 which represents a relatively narrow distribution.

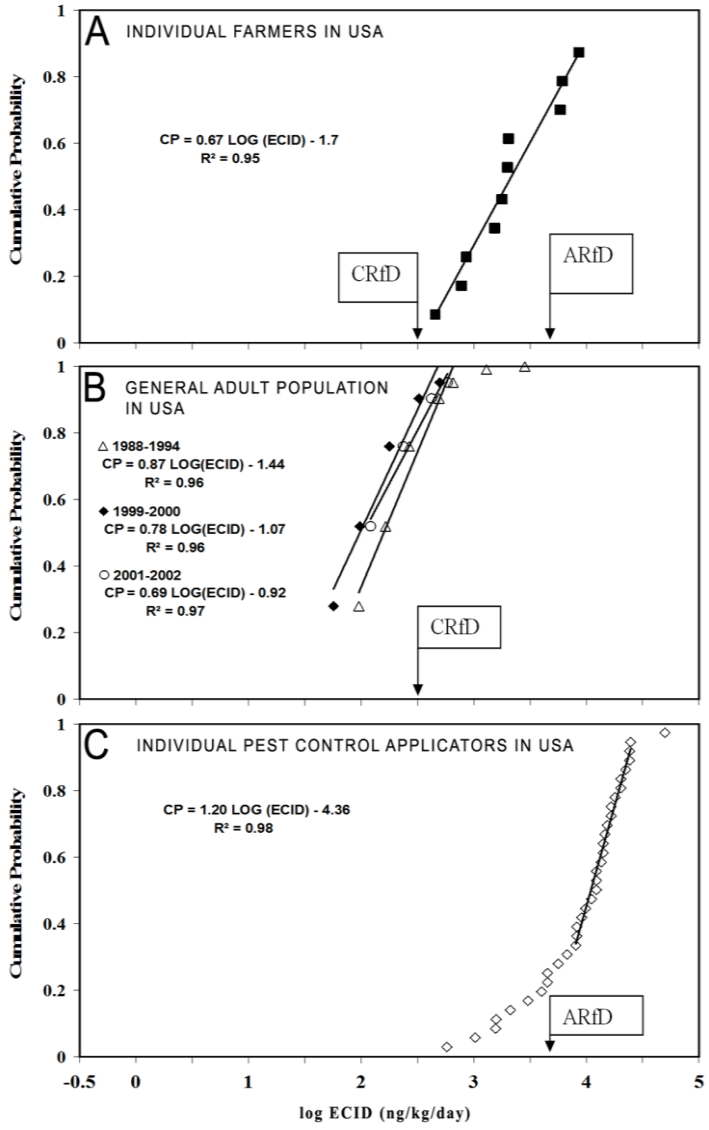
The USA farmers were reported to use ground booms and tractor-drawn spreaders which gives less chance of direct contact with the pesticide. Scher *et al.* [26] reported that some farmers were excluded from the study since they operated from an enclosed cab while spraying the pesticide. Further Scher *et al.* [26] reported that the usage of granular formulations would be expected to result in less exposure.

3.1.2. General Adult Population

The National Health and Nutrition Examination Survey (NHANES) was carried out by the Centers for Disease Control and Prevention in USA to monitor the levels of selected chemicals in urine from the population. The participants were males and females in different age categories resident in different areas of the country. For the analysis of chemicals, including TCP, a *spot* urine sample was obtained from each volunteer [22]. In the 1988–1994 survey the TCP concentrations of nearly

1000 participants from 26 locations with ages from 20 to 59 years were reported [30]. The detected occurrence of TCP in the urine of participants was at a level of 82%. In the 1999–2000 survey average TCP concentrations of randomly selected adults (832) between 20 and 59 years were reported [31]. The frequency of detections was 89% however the detection limit of 400 ng/L TCP was lower than that of the 1988–1994 survey (1000 ng/L). In the 2000–2001 survey the TCP levels of 1113 participants were reported [18].

Figure 2. Cumulative probability distribution (CPD) for the Equivalent Chlorpyrifos Ingested Dose (ECID) with the following: (**A**) Individual farmers in the USA after two spraying events in 2000–2001; (**B**) Adult population aged from 20 to 59 years in the USA from 1988 to 2002 reported in the National Health and Nutrition Examination Survey; (**C**) Individual pest control applicators from North Carolina, USA in 1998 (see Section 3.1).



The TCP levels were converted into ECIDs and CPD plots made as shown in Figure 2B. Little difference was observed between the distributions in the three surveys as reflected by the slopes of 0.9, 0.8 and 0.7 in 1988–1994, 1999–2000 and 2001–2002 respectively. But overall the highest ECID levels were observed during the 1988–1994 period and the lowest in 1999–2000.

Chlorpyrifos was introduced as an alternative to chlordane in indoor pest control during 1988–1994, resulting in a higher frequency of exposure [30,32]. Indoor exposure was assumed to be one of the major pathways of exposure in the general population in the USA. Relatively heavy usage of chlorpyrifos was recorded in the late nineties estimated at 9 to 14 million kg for agricultural and nonagricultural pest control purposes [32] while this was reduced to 5 million kg in early 2000 [18].The reduction of chlorpyrifos usage is probably reflected in the distributions from 1988 to 1994 onwards.

The reasons for a lower frequency of detections in NHANES 1999–2000 were discussed in Barr *et al.* [31]. It was suggested it occurred since there were major changes in the regulations related to chlorpyrifos which resulted in a reduction in usage. In addition there were differences in the study population from that of 1988–1994. Regulatory decisions were taken by USEPA to reduce indoor treatment with chlorpyrifos except for ant and roach baits [33]. Use in termite control at the pre and post construction stages of houses were prohibited by the end of 2005 with a successive phasing out over the previous years. This may have decreased the frequency of exposure among the general public. However the reason for slightly higher ECID levels during 2001–2002 as compared to 1999–2000 is not clear.

3.1.3. Pest Control Applicators

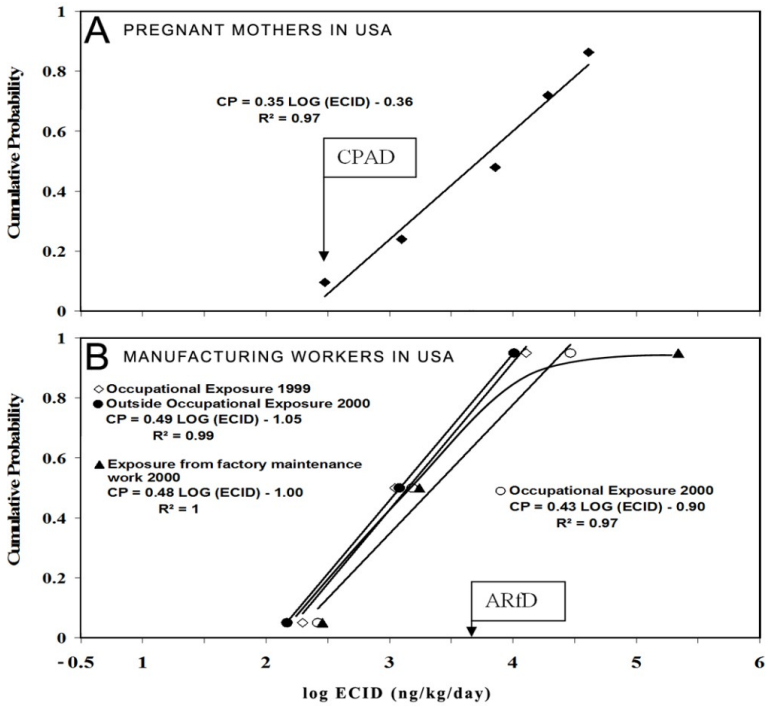
Chlorpyrifos exposure was assessed in 1998 with a group of termiticide applicators in the Piedmont region of North Carolina, USA [32]. The participants were thirty five volunteers between 18 and 54 years of age working as full time licensed applicators applying chlorpyrifos in houses. This study describes the details of chlorpyrifos usage by duration, extent and the amounts used as well as the concentrations of chlorpyrifos in breathing air. Hines *et al.* [32] monitored TCP in urine samples collected from the workers and presented as creatinine adjusted mean levels. In this current study, the TCP levels were converted to ECID and plotted as a CPD (Figure 2C) together with the ARfD for comparative purposes (see Table 1). The ECID levels were distributed in between 580 (2.8 log scale) and 50,000 ng/kg/day (4.7 log scale, Figure 2C). The slope of the CPD plot in the linear range was 1.2. However a broader distribution was observed below the linear range (2.7 to 3.8 log scale Figure 2C). This may be due to a group of applicators who are less active than would be expected and so exhibit less exposure.

3.1.4. ECID in USA Pregnant Mothers and Children

The exposure to pesticides was assessed in healthy pregnant mothers registered in maternity clinics in New York from 1998 to 2001 [34]. The participants were a group of 365 individuals, of diverse ethnicities, aged around 20 to 30 years with different educational backgrounds and recruited in early pregnancy. *Spot* urine samples were obtained from the mothers in their last months of the pregnancy for TCP analysis. The individual TCP levels were not reported but the creatinine adjusted TCP levels were reported at various percentiles and converted to ECID levels (Section 2.2) and are presented in Figure 3A. The ECID were distributed in a relatively wide range from 30 to 5200 ng/kg/day (1.5 to 3.7 log scale) with a slope of 0.4 (Figure 3A). The highest level at the 90% probability level is over

170 times than the lowest at the level of 10% probability. This distribution probably reflects the wide range of conditions and circumstances under which exposure occurs.

Figure 3. Cumulative probability distributions (CPD) for the ECID with the following: (**A**) Pregnant mothers aged about 20 to 30 years during 1998 to 2001 from New York, US (results from 365 individuals reported at the 10, 25, 50, 75 and 90 percent levels) (see Sections 2.2 and 3.1); (**B**) Manufacturing workers in the USA on four occasions with 50 to 53 individuals evaluated on each occasion during 1999 to 2000 (results reported for the 5, 50 and 95 percent levels (see Sections 3.1).



Indoor pesticide usage was assumed to be the most common source of chlorpyrifos exposure, according to a questionnaire answered by the participants [34]. Indoor pesticide usage, not exclusively chlorpyrifos, in bait traps, can sprays, gels, boric acid, sticky traps and pest bombs was reported by 72% of the 365 mothers. It was revealed that the relatively highly educated mothers had the highest TCP levels in their urine. However none of the other sociodemographic factors had any consistent relationship with the TCP levels. In addition the potential exposure by other sources such as diet, work place and outdoor environment were not assessed.

Several authors [35,36] have reported on the monitoring of chlorpyrifos in prechildren in the USA. TCP in urine was used which was found not to be a reliable guide to exposure if several sources are involved but exposure to chlorpyrifos and TCP from several sources and through several pathways and routes were identified. Rauh *et al.* [37] in a longitudinal study have reported that prenatal exposure to chlorpyrifos was associated with neurodevelopmental problems at the age of 3 years and deficits in memory and IQ at 7 years. The USEPA review of the registration of chlorpyrifos [29] recommended the cancelling of uses in schools and parks where children may be exposed.

3.1.5. ECID in Manufacturing Workers from USA

The impact of occupational chlorpyrifos exposure on the urinary TCP levels and blood ChE levels of the workers in a chlorpyrifos manufacturing plant in USA was reported by Garabrant *et al.* [4]. The ChE levels were analysed in blood together with the TCP levels in urine, obtained on four occasions, with 50 to 53 individuals, during 1999 and 2000. The TCP data was converted to ECID but the results were not reported on individuals but as the 5th, 50th and 95th percentiles which are plotted in Figure 3B.

A considerable difference in the ECID levels can be seen at the 0.95 probability level. The highest ECID was observed in the period when the workers were undertaking factory maintenance work (210,000 ng/kg/day, 5.3 log scale, Figure 3B). This was 21 times higher than the lowest ECID observed at the same probability level. This is most likely due to activities such as cleaning and repairing of equipment which may expose workers to high levels of chlorpyrifos.

3.1.6. Overview of Chlorpyrifos Exposure in Populations of the USA

An overview of the observed ECID levels in the US population is contained in Table 2. All of the ECID—CPD plots were approaching linearity (R^2 0.95 to 1.00) suggesting that the statistical distributions were approaching normal. The slopes of these CPD plots are indicative of the range of the exposures to chlorpyrifos in the population. The CPD distribution with the pesticide applicators had the highest slope (1.2) indicating a relative narrow range of exposures reflecting limited and consistent application behaviour within this group. On the other hand the pregnant mothers had the lowest slope (0.4) suggesting the widest diversity of exposure behaviours. The ECID levels for the whole population varied over a wide range from 500 to 210,000 ng/kg/day at the 0.95 level of exposure (see Table 2). The lowest levels were generally observed with the adults who were reported to have only nonoccupational exposure. The highest dose (210,000 ng/bw/day at 0.95 cumulative probability) represented an unusually high exposure situation in an occupational environment with manufacturing workers carrying out maintenance operations.

Table 2. Overview of the ECID in various USA populations at 0.95 Cumulative Probability Exposure.

Population	ECID (ng/kg/day)	CPD Plot characteristics [a]		
		Slope	Intercept	R^2
Farmers (2000–2001)	8.4×10^3	0.7	−1.7	0.95
General population adults				
1988–1994	0.6×10^3	0.9	−1.4	0.97
1999–2000	0.5×10^3	0.8	−1.1	0.96
2000–2001	0.6×10^3	0.7	−0.9	0.98
Pest control applicators (1998)	26.0×10^3	1.2	−4.3	0.98
Manufacturing workers (1999–2000)				
Low exposure	10.0×10^3	0.5	−1.0	1.00
High exposure	210.0×10^3	NA [b]	N/A	N/A [b]
Pregnant mothers (1998–2001)	5.0×10^3	0.4	−0.4	0.97

[a] Cumulative Probability = (slope) (log ECID) + intercept; [b] NA—Not Available.

The exposure levels with the manufacturing workers in normal working environments (10,000 ng/kg/day at 0.95 probability) were comparable with those of the farmers who were

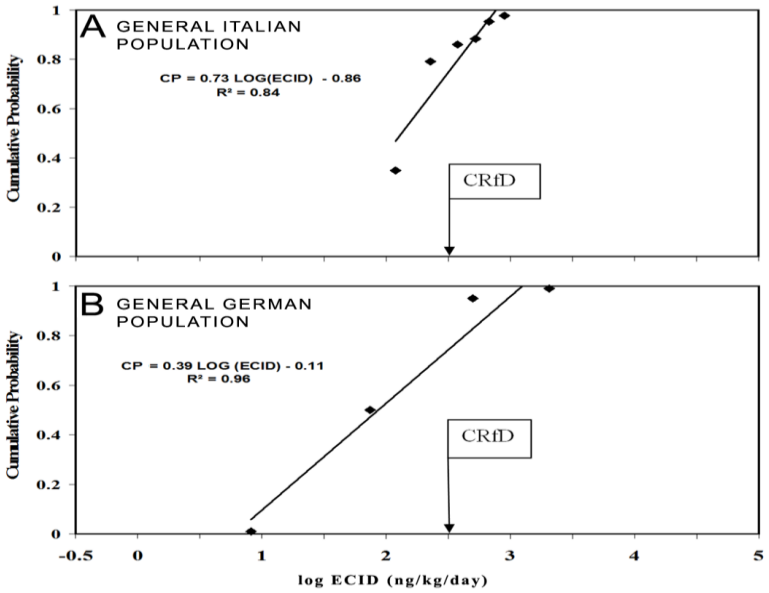
applicators (8400 ng/bw/day). The applicators showed a higher ECID level than in the nonapplicator farmers reflecting their greater involvement with activities related to pesticides. The pregnant mothers showed higher dose levels than were observed in the general adult population suggesting an unusually high exposure in a nonoccupational environment. However the physiological changes that may occur during pregnancy also might have an effect on the rate of creatinine excretion in the pregnant mothers and if so could affect the creatinine adjustment of the chlorpyrifos biomarker (TCP).

3.2. Equivalent Chlorpyrifos Ingested Doses (ECID) with Populations in Europe

3.2.1. ECID in the General Adult Population in Italy

Aprea *et al.* [38] assessed the urinary TCP levels in a group of Italian adults (42) with relation to their dietary habits during 1997. The study objectives were to evaluate pesticide exposure resulting from wine and food consumption with the general population. The participants were healthy males and females in the age range of 20 to 60 years, from the Pavia, Siena and Trento regions in Italy and had history of chlorpyrifos exposure. *Spot* urine samples were analysed for TCP and 88% of the samples had detectable TCP and the creatinine adjusted TCP concentrations were presented as ranges. In this current investigation the mean ECID levels were calculated (see Section 2.2) and plotted in Figure 4A. The observed levels were distributed in a range from 120 ng/kg/day (2.1 log scale) to 900 ng/kg/day (2.95 log scale) with a slope of 0.7. However 12% of the participants had no detectable TCP which means that the actual range of levels was from effectively zero to 900 ng/kg/day.

Figure 4. Cumulative probability distributions (CPD) for the ECID with the following: (**A**) 42 individuals in the general Italian population in the Pavia, Siena and Trento regions during 1997 (results reported for the ranges of TCP concentrations; (**B**) 50 individuals in the general German population of Meklenburg-Vorpommern regions (results reported at the maximum, median and 95 percent value) (see Sections 3.2).



A similar study was carried out by Saieva *et al.* [25] with 69 participants from two regions (Florence and Ragusa) in Italy during 1998. The participants had no history of chlorpyrifos exposure and supplied urine samples for the TCP analysis. The creatinine adjusted TCP levels were reported from which the corresponding ECID levels for the minimum, maximum and mean were calculated (see Section 2.2). The ECID levels were 60 ng/kg/day, 1300 ng/kg/day and 270 ng/kg/day (1.8, 3.1 and 2.4 log scale, data not plotted). These levels are comparable with the ECIDs calculated in the study of Aprea *et al.* [38] (Figure 4A).

In both studies it was suggested that the exposure is most likely to result from dietary intake. In addition Aprea *et al.* [38] reported that wine consumption had a significant effect on TCP levels in urine, which would be reflected in the ECID levels. Saieva *et al.* [25] found a relationship between smoking and high TCP levels in the study group. However, neither of the studies suggests significant exposure through indoor pesticide treatment since this is uncommon in Italy.

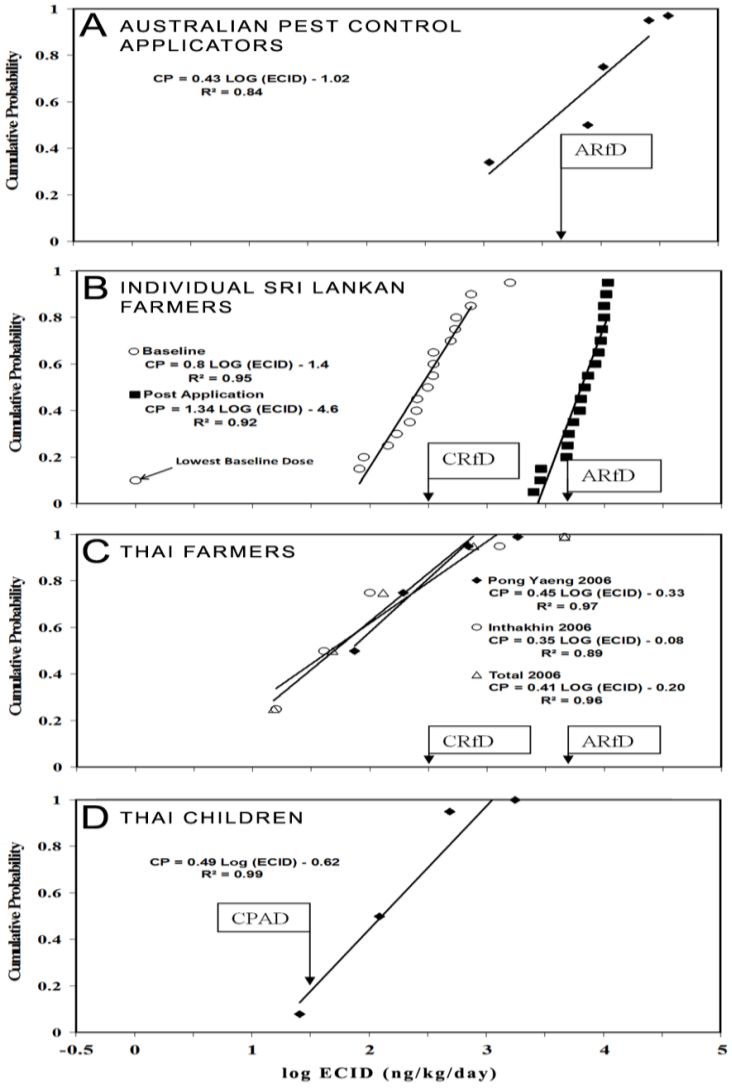
3.2.2. ECID in German General Adult Population

Koch *et al.* [39] assessed the TCP levels in urine from fifty adults in the general population of Meklenburg-Vorpommern in Germany. It was assumed that the TCP levels observed resulted from the intake of food treated with chlorpyrifos and chlorpyrifos methyl pesticides. However, the usage of these pesticides in Germany was not reported. The participants who supplied *spot* urine samples for analysis were men and women aged 22 to 57 years who had never experienced occupational exposure to organophosphate pesticides. TCP was detected in all the urine samples. The creatinine adjusted minimum and maximum TCP levels together with the levels for the median and 95th percentile were reported. The TCP levels were converted into ECID (see Section 2.2) and these are presented in Figure 4B. The levels ranged from 0.8 to 2100.0 ng/kg/day (0.9 to 3.3 log scale), with a mean dose of 160.0 ng/kg/day (2.2 on log scale) and the CPD had a slope of 0.4 (see Figure 4B).

3.3. Equivalent Chlorpyrifos Ingested Doses (ECID) in Australian Pest Control Applicators

During 1998 and 1999 Cattani [40] evaluated the workplace exposure to chlorpyrifos, in a group of pesticide applicators from Perth, Australia. The participants were from a number of licensed pest control companies and had volunteered to participate. Some workers used chlorpyrifos and bifenthrin for termite control purposes while the others used only chlorpyrifos. All were involved in pre-construction, post-construction and underfloor treatments of buildings using similar equipment. Chorpyrifos levels were measured in breathing air and surface wipe samples together with urinary TCP and blood ChE levels. The urine samples were collected from workers (19) before and after a chlorpyrifos application event and were analysed for TCP. The creatinine adjusted TCP concentrations were reported for the minimum, maximum, median, 75th and the 95th percentiles. The post-application TCP levels were converted to ECID levels (see Section 2.2) and are presented in the Figure 5A. The CPD had a slope of 0.4 and the levels ranged from 1100 to 37,000 ng/kg/day (3.0 to 4.6 log scale). The highest ECID was 33 times greater than the lowest level.

Figure 5. Cumulative probability distributions (CPD) for the ECID with the following: (**A**) 19 Australian pest control applicators after an application event during 1998 to 1999 (results reported as minimum, maximum median, 75 and 95 percent levels. (see Section 3.3); (**B**) 19 individual Sri Lankan farmers at baseline and post application exposure during 2000. (see Section 3.4); (**C**) 136 male Thai farmers during 2006 (results reported at the maximum, 25, 50, 75 and 95 percent value) (see Section 3.5); (**D**) 207 Thai children during 2009 (results reported at the maximum, minimum, median and 95 percent value) (see Sections 3.5).



3.4. Equivalent Chlorpyrifos Ingested Dose (ECID) in Sri Lankan Farmers

Aponso and Manuweera [2] conducted a study to assess chlorpyrifos exposure during a chlorpyrifos spraying event with a group of individual farmers (19) in the Kandy district of Sri Lanka during 2000. The farmers were spraying an overhead canopy with hand operated spraying equipment. The ECID in urine was calculated prior to commencing a typical chlorpyrifos spraying event (pre-exposure) as well as throughout and after the event (post exposure) (see Section 2.2). The pre-exposure data represents the baseline exposure of farmers to chlorpyrifos with additional exposure occurring during spraying

events. The farmers had not used chlorpyrifos for at least ten days prior to the study and therefore the baseline TCP in the farmers indicates the exposure to chlorpyrifos from routes other than the spraying event. The CPD plots of this data are shown in Figure 5B.

The doses are in between 1.0 ng/kg/day (0.0 log scale, Figure 5B) and 1600.0 ng/kg/day (3.2 log scale, Figure 5B). The median ECID is 379.0 ng/kg/day (2.6 log scale, Figure 5B) and the slope of the distribution plot is 1.3. The post application ECID levels were distributed in between 2500 and 11,000 ng/kg/day (3.4 and 4.0 log scale, Figure 5B). The slope of the post application CPD was 1.3 as compared to the baseline plot which was 0.8.

3.5. Equivalent Chlorpyrifos Ingested Doses (ECID) in Thai Farmers and Children

3.5.1. ECID in Farmers

Farmers (males, 136 in total) from Chang Mai, Thailand in two communities (Pong Yaeng and Inthakhin) involved in mixed crop cultivation participated in a pesticide exposure evaluation study during 2006 [41]. A number of pesticides were used during the three months prior to the study with chlorpyrifos being the most common. All the farmers used back pack reservoirs with hand pumps to apply the pesticides.

A *spot* urine sample was collected from each farmer during the study period for TCP analysis. The creatinine adjusted TCP concentrations were reported at maximum, 25th, 50th, 75th and the 95th percentiles. About 77% of the farmers had detectable TCP levels which were converted into ECID (see Section 2.2) and are plotted as a CPD in Figure 5C.

The ECID in Pong Yaeng and Inthakhin farmers [41] ranged from 70.0 to 1800.0 ng/kg/day (1.9 to 3.3 log scale, Figure 5C) and 20.0 to 4600.0 ng/kg/day (1.2 to 3.7 log scale, Figure 5C). At 0.95 cumulative probability levels the farmers from Inthakhin region showed more elevated levels of ECID than the farmers from PongYaeng. Panuwet *et al.* (2008) reported that the cropping pattern selected by the farmer has an influence on exposure [41].

3.5.2. ECID in Children

Panuwet *et al.* [42] analysed the urine samples from a group of school children (207) in Chiang Mai, Thailand aged between 12 to 13 years who were identified as having agricultural and nonagricultural family backgrounds. *Spot* urine samples were analysed for TCP and 92% had detectable levels. The creatinine adjusted TCP levels were reported for the minimum, maximum, median and the 95th percentile. In the present study the TCP levels were converted to ECIDs (see Section 2.2) and are presented in Figure 5D. The ECID ranged from 30.0 to 1800.0 ng/kg/day (1.4 to 3.2 log scale) with the highest from students in an agricultural environment. Dietary exposure was assumed to be the main pathway of exposure with chlorpyrifos being monitored frequently in dietary components in Thailand [42]. It is interesting to contrast this result with that of children in Costa Rica who live in plantations where bags treated with chlorpyrifos are used to protect fruit.

3.6. Equivalent Chlorpyrifos Ingested Doses (ECID) in Vietnamese Farmers

Chlorpyrifos is the most common pesticide used in Vietnam for rice cultivation. Phung *et al.* [12] collected urine samples (108) both before and after a spraying event from farmers (18) who were pesticide applicators using back pack hand operated sprays. TCP levels were estimated and the ECIDs were calculated using creatinine adjustment. The baseline exposure levels ranged from 30 to 1980 ng/kg/day while the post application levels were 350 to 94,000 ng/kg/day and the data were plotted as CPDs [13]. Exposure at the 0.95 and the 0.50 levels were reported for the baseline (1600 and 30 ng/kg/day respectively) and the post application situations (11,000 and 680 ng/kg/day respectively) (Table 3).

Table 3. Equivalent chlorpyrifos ingested dose (ECID, ng/kg/day) for farmers from various countries.

Cumulative Probability Level	Sri Lankan Farmer-Baseline, 2000 [a] (log dose)	Sri Lankan Farmer-Post Application, 2000 [a] (log dose)	Thai Farmer, 2006 [b] (log dose)	US Farmer–Post Application, 2000-2001 [c] (log dose)	Vietnam Farmer–Baseline, 2011 (log dose)	Vietnam Farmer–Post Application, 2011 (log dose)
0.50	$0.3\ 2 \times 10^3$ (2.5)	6.8×10^3 (3.8)	0.05×10^3 (1.7)	2.0×10^3 (3.3)	0.24×10^3 (2.4)	19.4×10^3 (4.3)
0.95	1.6×10^3 (3.2)	11×10^3 (4.0)	4.6×10^3 (3.6)	8.4×10^3 (3.9)	9.0×10^3 (3.95)	97.7×10^3 (5.0)

[a] see Figure 5B; [b] see Figure 5C; [c] see Figure 2A.

4. Overview of Equivalent Chlorpyrifos Ingested Dose (ECID) in Similar International Populations

4.1. Farmers from Sri Lanka, Thailand, USA and Vietnam

The ECID levels for the farmers from Sri Lanka, Thailand, USA and Vietnam were compared at the median (0.50 probability) and high exposure (0.95 probability) levels (see Table 3). It is noteworthy that with the Sri Lankan and Vietnamese farmers the post application levels of ECID (see Figure 5B, Table 3) showed a major increase over the baseline at the 0.95 and the 0.05 levels , ×7 to ×22 (Sri Lankan) and ×11 and 81 (Vietnam).

The baseline levels were estimated immediately before chlorpyrifos application and were not related to pesticide application [2]. It was assumed to be derived from the diet containing contaminated plants and other non-agricultural sources. The median baseline levels (0.50 level , Table 3) with all the farmers were relatively low (0.3×10^3, 0.05×10^3, 2.0×10^3, 0.24×10^3, 19.4 ng/kg/day) and within a somewhat similar range, except for the Vietnamese farmers, and there was also a similar limited range of values at the 0.95 level (1.6×10^3, 4.6×10^3, 8.4×10^3, 9.0×10^3, 92.7 ng/kg/day) again with the exception of the Vietnamese farmers.

The ECID with the Thai farmers was probably a reflection of the exposure from various sources including those which occurred during their normal farming activities [41]. However the post application ECIDs were directly related to the chlorpyrifos exposure received from a planned application event [2,13]. In contrast, the Thai farmers exposure was not related to an application event and thus could be expected to be lower [41]. In addition chlorpyrifos was not one of the frequently used pesticides with the Thai farmers.

The USA farmers were shown to have a post application ECID of 8.4×10^3 ng/kg/day at the 0.95 probability level (see Table 3 and Figure 2A) which was below the level of the Sri Lankan and Vietnamese farmers (see Table 3 and Figure 5B). Most of the Sri Lankan and Vietnamese farmers were not using personal protective equipment and had a greater potential for exposure due use of personal individual hand operated sprayers [2]. In contrast, USA farmers used ground booms and tractor mounted spreaders [26] which provide a higher level of protection to the applicator.

4.2. Pest Control Applicators and Manufacturing Workers in Australia and USA

The Equivalent Chlorpyrifos Ingested Dose (ECID) levels for pest control applicators and manufacturing workers in the USA and Australia were compared at the 0.50 probability and 0.95 probability levels (see Table 4). The USA manufacturing workers have a differing exposure depending on the working cycle at the manufacturing plant. At a low exposure period in the working cycle the exposure at the 0.50 level is 1.1×10^3 ng/kg/day while at the 0.95 level it is 10.0×10^3 ng/kg/day (see Table 4). However at the 0.95 probability level there is a much higher level of exposure due to the annual factory shutdown and maintenance period (210×10^3 ng/kg/day, see Table 4) [4]. Interestingly this difference was not observed at the 0.50 probability level with 1.1×10^3 at median exposure and 1.8 ng/kg/day at high exposure (Table 4).

Table 4. Equivalent chlorpyrifos ingested doses (ECID) in pest control applicators and manufacturing workers in Australia and USA.

Cumulative probability level	US pest control applicators (1998) [a] (ng/kg/day) (log dose)	US manufacturing workers (1999–2000) [b] (ng/kg/day)		Australian pest control applicators (1998–1999) [c] (ng/kg/day) (log dose)
		Low Exposure (log dose)	High Exposure (log dose)	
0.50	12.0×10^3 (4.1)	1.1×10^3 (3.04)	1.8×10^3 (3.2)	7.8×10^3 (3.9)
0.95	26.0×10^3 (4.4)	10.0×10^3 (4.0)	210.0×10^3 (5.3)	26.0×10^3 (4.4)

[a] see Figure 4; [b] see Figure 3A; [c] see Figure 5A.

Pest control applicators in both Australia and the USA were reported to be involved with termite control work using chlorpyrifos [32,40]. Termiticide application normally is a full time occupation carried on throughout the working week, which necessitates handling of pesticides frequently. For example USA applicators were reported to be working more than five days a week during busy periods [32]. In addition most of the applicators were operating in enclosed crawl spaces with comparatively low ventilation. Cattani [40] has described the protective measures taken by the applicators but believed to be insufficient to prevent significant exposure.

4.3. General Populations of Europe, Sri Lanka, Thailand, USA and Vietnam

A comparison was made of the international general populations of adults and some population groups, specifically pregnant mothers in USA and children in Thailand (see Table 5). It is interesting to note that the ECIDs of the general populations of Europe and USA, as well as the baseline level in Sri Lankan and Vietnamese farmers and Thai children, at both the median (0.5 probability) and the

high exposure (0.95 probability) levels (0.1 to 0.8 × 10^3 ng/kg/day and 0.5 to 9.0 × 10^3 ng/kg/day respectively) are similar with all values falling within a relatively narrow range. In these populations there is an absence of direct known sources of exposure.

The adults in USA would be expected to be exposed mainly from indoor environments treated for household pests and the diet [30,31]while with the Italian and German populations the exposure was believed to be through the diet [38,39] . None of the adults were known to have occupational or any other known exposure to the pesticide.

The Thai children were representative of children in agricultural and non- agricultural families [42]. It was assumed that the most common pathway of exposure was through the diet, which was suggested by the frequent detection of chlorpyrifos in food commodities [42]. However in the estimation of ECID in children, the common physiological parameters were used which were believed to be appropriate for the age group (average body weight and average daily creatinine excretion rate (Section 2.2).

The highest ECIDs, among the general populations which was not exposed directly, were observed in the USA pregnant mothers (see Table 5) which was 0.8 × 10^3 ng/kg/day at the median level (0.5 probability) and 5.0 × 10^3 ng/kg/day at the high exposure level (0.95 probability) (see Table 5 and Figure 3). Most of the mothers were believed to be mainly exposed from indoor usage of household pest control devices [34]. However the dietary and other pathways would also be expected [31]. Nevertheless the levels observed with the mothers are unusual.

Table 5. Equivalent Chlorpyrifos Ingested Doses (ECID) in General and Some Specific Population Groups.

Cumulative Probability Level	Sri Lankan Farmer–Baseline (2000) [a] (ng/kg/day) (log Dose)	USA General Population [b] (2001–2002) (ng/kg/day) (log Dose)	European General Population [c] (ng/kg/day) (log Dose)		USA Pregnant Mothers [d] (1998–2001) (ng/kg/day) (log Dose)	Thai Children [e] (2009) (ng/kg/day) (log Dose)	Vietnam Farmer–Baseline (2011) (ng/kg/ day) (log dose)
			Italy (1997)	Germany (2001)			
0.50	0.3 × 10^3 (2.5)	0.1 × 10^3 (2.0)	0.1 × 10^3 (2.0)	0.1 × 10^3 (2.0)	0.8 × 10^3 (2.9)	0.24 × 10^3 (2.4)	0.1 × 10^3 (2.0)
0.95	1.6 × 10^3 (3.2)	0.6 × 10^3 (2.8)	0.6 (2.8)	0.5 (2.7)	5.0 × 10^3 (3.7)	9.0 × 10^3 (3.95)	0.5 × 10^3 (2.7)

[a] see Figure 5B; [b] see Figure 2B; [c] see Figures 4A (Italy) & 4B (Germany); [d] see Figure 3B; [e] see Figure 5D.

5. Risk Characterisation using the Hazard Quotient (HQ)

5.1. Background

Guidelines have been developed by various bodies for the evaluation of the adverse health effects due to chlorpyrifos (Table 1). Since the USEPA Guideline Values (GVs) are the most comprehensive it was decided to use these in this investigation. These guidelines also give a common basis for the comparison of health risk in different situations and in different countries. There are included specific guidelines for different population groups (Table 1). Risk to health can be evaluated by calculation of the Hazard Quotient (Exposure Dose/Guideline Value) using the GVs. The guidelines are comprised of the Acute Reference Dose (ARfD) which is used for high level short term exposure in occupational and similar situations; the Chronic Reference Dose (CRfD), applicable to low level repeated exposure

usually with nonoccupational situations; the Chronic Population Adjusted Dose (CPAD) for chronic exposure of sensitive populations (females of child bearing age, infants and children[1]). The GVs of ARfD, CRfD and CPAD as well as the ECID are all in units of ng/kg/day and represent the dose ingested in food and water per day (see Table 1).

5.2. Hazard Quotients (HQ) Calculated Using the US EPA Guideline Values (GVs)

The HQs of the nonoccupationally exposed populations were calculated at the 0.95 ($HQ_{0.95}$) and 0.50 ($HQ_{0.50}$) levels using the USEPA Chronic Reference Dose (CRfD). At the 0.50 level the populations of Sri Lankan, Vietnamese and Thai farmers as well as the general populations of USA, Italy and Germany had an acceptable risk with HQs of unity or less. A relatively low potential risk was observed at the 0.95 probability in the general populations of USA, Italy and Germany with $HQ_{0.95}$ from 1.6 to 2.0. The Sri Lankan and Vietnamese farmers at baseline exposure had a higher risk with a $HQ_{0.95}$ of 5.3 and 30.

The Hazard Quotients (HQ) of the occupationally exposed populations (Table 6) were calculated using the ECIDs at high exposure (0.95 probability, $HQ_{0.95}$) and median exposure (0.50 probability levels, $HQ_{0.50}$) using the Acute Reference Dose (ARfD) of the USEPA (Table 1). The HQs range between 0.2 to 3.9 for the median exposure group, while the high exposure group ranged from 1.7 to 42.0. The highest risk of potential adverse effects was observed with the high exposure group (0.95 probability) with the USA manufacturing workers with $HQ_{0.95}$ of 42.0 and the Vietnamese farmers at $HQ_{0.95}$ of 19.5. The Sri Lankan and Vietnamese farmers and the pest control applicators in USA and Australia were at a relatively lower risk at the median level (0.50 probability) with $HQ_{0.50}$ of 1.3, 3.9, 2.4 and 1.5 respectively (Table 6). The USA farmers were at relatively low risk at the 0.95 probability level with a $HQ_{0.95}$ of 1.7 (See Table 6) and a $HQ_{0.50}$ of 0.4.

Table 6. Hazard Quotients (HQ) for occupationally exposed populations based on the ARfD [a].

Cumulative Probability	Hazard Quotients (HQ)						
	Sri Lankan farmer-Post Application [b]	US farmer [b]	US pest control applicator [c]	US manufacturing worker [c]		Australian pest control applicator [c]	Vietnamese farmer-Post Application [b]
				Low Exposure	High Exposure		
0.50	1.3	0.4	2.4	0.2	0.3	1.5	3.9
0.95	2.2	1.7	5.2	2.6	42.0	5.2	19.5

[a] see Table 1; [b] see ECID in Table 3; [c] see ECID in Table 4.

The HQs for the more sensitive population groups were calculated with the Chronic Population Adjusted Dose (CPAD) (Table 1) for pregnant females and children having repeated exposure. The USA mothers were at high risk having the highest $HQ_{0.95}$ of 173 and a $HQ_{0.50}$ of 26.6 both representing a high exposure. The Thai children have a comparatively lower risk having an $HQ_{0.50}$ of 3.3 and a $HQ_{0.95}$ of 16.6. It is interesting to contrast this result with that of children in Costa Rica who live in plantations where bags treated with chlorpyrifos are used to protect fruit. With more than half the children their estimated intake dose exceeded the CPAD and some also exceeded the ARfD and the CRfD [43]. In Jianjsu, China, a survey of urinary TCP in 2 year old children revealed that the TCP occurred in 70% of the children. The $HQ_{0.75}$ was 2.5 suggesting a lower level of risk than the Thai

children and the other groups mentioned above [44]. However the CPAD value would require the use of appropriate biological data to confirm the health risk represented by these HQs.

6. Conclusions

These results indicate that chlorpyrifos residues are widely distributed in the global human population. For example, the general population in the USA from 1988 to 2001 had detectable occurrence of residues in 82% to 89% of individuals. The general population in Germany in 2001 had detectable residues in the whole population and Italy, in 1997, in 88% of the population. This exposure is believed to result from pesticide treatment of crops and the resultant occurrence of residues in consumed food, rather than occupational exposure. It resulted in HQ_{50} values of less than unity and thus not considered to be a public health risk to the global population.

However, there are some specific population groups which have considerably higher exposure than the general population groups. For example, the ECID levels in pregnant mothers in USA during 1998 to 2001, at median exposure, were 26.6 times the exposure represented by the CPAD.

Also, high levels of risk, exceeding ARfD, were apparent in the high exposure group (0.95 level) with the occupationally exposed groups of manufacturing workers in 1999 to 2000 ($HQ_{0.95}$ 2.6 to 42.0) and the pest control applicators in Australia (1998 to 1999), and USA (2000 to 2001) ($HQ_{0.95}$ 5.2). Farmers had a high level of risk at the high exposure level (0.95 level) when the HQ was calculated using the ARfD. Those from Sri Lanka (2000) and Vietnam (2011), after a spraying application, had $HQ_{0.95}$ at 2.2 and 19.5 respectively; Thailand (2006) at $HQ_{0.95}$ of 15.3; and USA (2000 to 2001) at $HQ_{0.95}$ of 1.7.

This review demonstrates that chlorpyrifos exposure often occurs in human populations in levels which exceed the guidelines recommended by the USEPA. Some of the exceedances, and the derived HQs, are relatively high and indicate the possibility of adverse effects in the human populations affected. However, it should be noted that management practices and other factors may have led to changes in exposure since these investigations were made.

Conflicts of Interest

The authors have no conflict of interest.

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Author Contributions

This research was carried out while JM was a M.Phil. candidate at Griffith University with QY and DC as supervisors. JM carried out the work with the supervisors guidance while QY and DC prepared it for publication.

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RESEARCH ARTICLE

Chlorpyrifos contamination of fresh water in a commercial vegetable cultivation area in Sri Lanka and factors affecting contamination

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Abstract: The study investigated the interrelationships among chlorpyrifos (CPF) concentrations in water resources, CPF application and rainfall during peak pesticide application period with usual rainfall pattern. Water samples were collected at three day intervals from groundwater and surface water resources at Marassana, a commercial vegetable cultivation area in the Kandy District, Sri Lanka, during a 5-month period. CPF application and rainfall data were also collected simultaneously. High performance liquid chromatography analyses revealed that the average CPF concentration in groundwater and surface water samples were 0.63 and 0.52 µg/L, respectively. The respective corresponding maximum values were 7.1 and 3.7 µg/L. Multiple linear regression analysis of the data established that 1 L of CPF 40 % (= 400 g/L concentration) applied in the catchments increased the CPF concentration in the groundwater and surface water by 0.65 µg/L and 0.120 µg/L, respectively; 1 mm of cumulative rainfall received increased the CPF concentration of surface water by 0.021 µg/L but did not affect the groundwater concentration significantly. Uncertainties in the model parameters analysed using Monte Carlo stochastic simulation established that there was an 88 % probability for the CPF concentration to remain positive in the surface water.

Keywords: Chlorpyrifos, pesticide, pollution, uncertainty groundwater, surface water.

INTRODUCTION

Chlorpyrifos (CPF) is the common name used for *O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate, which is a broad-spectrum chlorinated organophosphate insecticide. CPF accounts for nearly 50 % of all the organophosphate insecticides imported, and about 20 to 25 % of all insecticides imported to Sri Lanka (Registrar of Pesticides, 2007). CPF and other pesticides applied in the field are likely to be transported from field to surface water mainly by surface runoffs and to groundwater by leaching through soil. The objective of this study was to estimate the CPF concentrations in surface water and groundwater in a commercial vegetable cultivation area in the central hills of Sri Lanka, and to determine the factors affecting the contamination. The factors considered were, quantities of CPF applied, rainfall in the study area, soil properties and the CPF mobility in soil.

CPF is a pesticide having a non-polar nature and it does not easily ionize in solution. Therefore, solubility of CPF in water varies within a low range, between 0.44 - 1.12 mg/L at a temperature range of 25 – 20 ^{o}C (Gebremariam, 2011). CPF is stable for several weeks in neutral or slightly acidic conditions when stored at room temperature (WHO, 2002). It is moderately toxic and classified in Toxicity Category II for all exposure routes (Smegal, 2000). According to the United States Environmental Protection Agency (USEPA, 1999), exposure to CPF could result in neurotoxicity in animals and humans and decreased birth weight of babies (Zhao *et al*., 2004 ; Tian *et al*., 2005), and increased risk of lung cancer (Dinham, 2005). The maximum permissible level for CPF in fresh water is 0.041 µg/L according to the USEPA (2009) water quality criteria. The 2008 amendment (NER, 2008) to the Sri Lankan National Environmental Regulations (NER) has increased the tolerance limit of total pesticides for the discharge of

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effluent into inland surface waters from "undetectable" (NER, 1990) to a maximum of 5 µg/L. The regulation stipulates that such discharged effluent should be diluted by at least eight volumes of clean receiving water, which amounts to 0.55 µg/L, the maximum permissible tolerance limit for pesticides in surface water. The permissible levels of CPF for ground water resources are not yet defined.

CPF has been found at concentrations bound by a maximum of 0.109 µg/L in four out of the 544 water samples collected from irrigation tanks and drinking water sources in the Dry Zone of Sri Lanka (Aponso *et al*., 2002). Water samples collected from a small stream running through a commercially cultivated area in Sri Lanka have been positive for CPF with the maximum of 2.48 µg/L (Menike *et al*., 2007; 2008), which is a many- fold increase above the tolerance limits stated by the Sri Lankan standard (NER, 2008). A number of international research studies have also reported detection of CPF residues in surface water, groundwater and soil (Fenelon & Moore, 1998; Troiano *et al*., 2001; Jergentz *et al*., 2005; Menon *et al*., 2005; Claver *et al*., 2006; Ntow *et al*., 2008; Muhamad *et al*., 2010).

Although numerous factors affect the pesticide concentrations in ground and surface water bodies, many of these factors such as soil type, stream channel network, land use, and landscape remain almost unchanged over time for a given geographical system. In such cases, changes of pesticide concentrations in surface water and groundwater are primarily dependent on factors such as rainfall, quantities of pesticide application in the catchments, soil properties and pesticide mobility in soil. Rainfall can be used as a proxy for the total amount of surface runoffs and pesticide application represents the source of contamination.

Mathematical models to predict pesticide occurrence at surface water bodies have been developed based on detailed descriptions of transport processes (Nakano *et al*., 2004; Leu *et al*., 2005). Uncertainties in modelling the complexity of natural systems have been observed to limit the predictive capacity of such models (Leiguo *et al*., 2004). Variations in pesticide concentrations in surface and ground waters with respect to parameters such as rainfall, transport and the amount of pesticide applied, have been established using multiple linear regression analysis (MLR) (Kreuger & Törnqvist, 2008; Sprague & Nowell, 2008).

A Sri Lankan commercial vegetable cultivation area where CPF is the most widely applied insecticide was chosen for this case study. Water samples were collected from selected surface water and groundwater locations in the study area. CPF concentrations of the water samples were determined using a high performance liquid chromatograph (HPLC). Statistical methodologies like, mean equality test and MLR were then used to analyse the interrelationship among CPF concentration, CPF application and rainfall, and to develop a linear regression model. The model was found to be statistically significant in the case of CPF contamination of surface water. Uncertainties surrounding the developed model were accounted for in the predictions made by a stochastic model developed using the Monte Carlo stochastic simulation procedure (Smith, 2002). In the case of groundwater, no significant statistical relationships were found. The hypothesized cause of groundwater contamination as CPF moving through the soil to reach groundwater was justified by the results (Menike *et al*., 2011).

METHODS AND MATERIALS

Study area

Figure 1 shows the schematic of the study area, which is a cultivated field of 35 acres in the catchments of a small stream, called Kiwullinda Oya running through Marassana in the Central Sri Lanka. The geographical coordinates of Marassana are 7° 13' 0" North, 80° 44' 0" East. The area belongs to the mid country Intermediate Zone, which receives an average annual rainfall of 1100 mm, of which, about 65 % is received from the north-east monsoon and the rest from the south-west monsoon (Punyawardhana, 2008).

The study area was divided into catchments A and B. Catchment A shown in Figure 1, was about 19 acres belonging to 41 farmers. About 65 % of the total land area was cultivated with tomato, 20 % with bitter gourd, snake gourd and loofah, and the other 15 % with cabbage, green chilli and root vegetables. Catchment B, as shown in Figure 1, was about 16 acres belonging to 27 farmers. About 70 % of this area was cultivated with tomato, 15 % with bitter gourd, snake gourd, green chilli and root vegetables, and the other 15 % with cabbage**.**

Properties of the soil at the area studied (Menike *et al*., 2011) were as follows: the soil structure was sandy clay loam; organic matter content (OM) was 3−3.2 %; P content was 60 – 150 ppm; K content was 160 – 400 ppm and the soil pH was in the acidic range, the maximum reported value being 6.4.

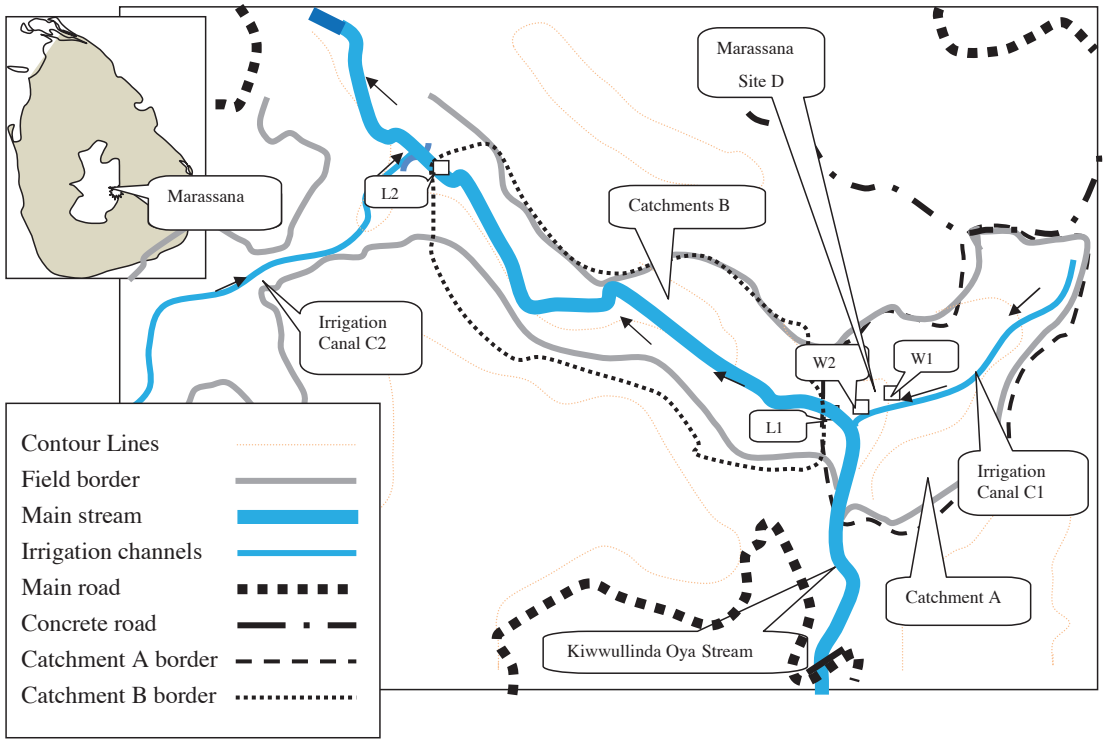

Figure 1: Schematic showing the sampling locations in relation to the site selected (not drawn to scale)

Water resources

Kiwullinda Oya stream was where the surface water resource studied. Water samples were collected at locations L_1 and L_2 in the stream (Figure 1). An irrigation canal (labelled C_1) receiving surface runoffs from catchment A merged with the stream just upstream of L_1. Irrigation canal (labelled C_2) situated outside the study area merged with the stream just downstream of L_2. Even though no irrigation canal merged with the stream in between L_1 and L_2, there was no hindrance for the surface runoffs from catchment B to reach the stream between L_1 and L_2 (as shown in Figure 1).

There were two community wells in the study area (W_1 and W_2) situated at the lowest elevation in catchment A. Those two wells were selected as the groundwater sources for the study. The irrigation canal C_1 ran adjacent to the walls of the wells (Figure 1). The depth and cross sectional area of well W_1 measured with 0.05 m accuracy, were 3 m and 2.5 m x 3 m, respectively, and those of W_2 were 2.5 m and 2 m x 3 m, respectively. Both wells were protected with 0.5 m of freeboard to prevent surface runoff water mixing with well water. Water level of the wells reached up to ground level during rainy season and decreased by 1.5 m from ground level in the drought season.

Sample collection and preparation

Water sample collection: The water samples were collected from L_1, L_2, W_1 and W_2 at a frequency of once in three days as described by Leiguo *et al*. (2004) during the vegetable cultivation period spanning five months. Water samples were collected in 500 mL amber glass bottles with glass lids, which were cleaned according to the procedure provided by Korth and Foster (1998). Sample collection from all four locations were carried out at about 7.00 am to minimize the mixing of sediments due to farmers' activities.

The water samples from the stream were collected manually by holding the immersed bottle open at a position within 3 cm deep from the top surface of the water flow. The samples from the wells were collected in bottles using a galvanized bucket. All the water samples were transported to the laboratory situated 40 km away from the research site, within 3 h of sample collection. The samples were stored below 4 ^{o}C temperature until the time of extraction.

Preparation of water samples: Sample preparation and analysis of CPF were carried out based on the methodology described by Korth and Foster (1998) and CIPAC Handbook 1C (1985). In this method, water samples from cold storage were equilibrated at room temperature and filtered using a standard code H20261 filter paper into a 1000 mL separating funnels, and 20 mL of HPLC grade dichloromethane (DCM) was added to each separating funnel. Funnels were shaken by inverting 180^0 while releasing the stopper from time to time to release the built up pressure. Shaking was repeated 50 times and the funnels were kept in a rack for 45 min for separation. Once the separation occurred the DCM with CPF at the bottom of the separating funnel was allowed to run into a 50 mL labelled round flask by opening the stopper at the bottom and the whole extraction procedure was repeated thrice. The solvent collected was concentrated and evaporated to dryness. The residue was dissolved in acetonitrile prior to the analysis using HPLC.

Analysis of samples

HPLC analysis for CPF: The sample residues were analysed for the presence of CPF using a HPLC (Agilent HP 1100 series, Chemstation). In this method, a C_8 column (ZORBAX Eclipse XDB-C8; 4.6 × 150 mm, 5 µm) was used for analysis under 62 bar pressure of at a wavelength of 285 nm in variable wavelength detector (VWD). The composition of the mobile phase was 60 % v/v HPLC grade acetonitrile, 39.5 % v/v deionised water and 0.5 % v/v HPLC grade acetic acid at a constant flow rate of 1 mL/min. A 20 µL aliquot of the sample was manually injected at a temperature of 25 ^oC. The HPLC run time was 20 min and the peak due to CPF on the chromatogram was identified at a retention time of 15.25 ± 0.30 min.

Quality control: Quantification of CPF was carried out using a calibration curve that was developed by injecting triplicates of 13 different concentration levels of CPF standards ranging between 0.0 and 700 μgL^{-1}. Linear relationships between the ratios of the peak area and the corresponding concentrations were observed with a correlation coefficient of 0.99, and a slope of 0.024. These values agree with the guidelines provided by Bassett *et al*. (2000). Coefficients of the linear regression were used in the respective conversion. The minimum level of detection of CPF was 0.001 µg/L, established according to the method described by Bassett *et al*. (2000). The repeatability (precision) is usually expressed as the relative standard deviation (RSD). The precision in terms of repeatability was obtained by carrying out the extraction and analysis of 12 different fortified samples. Each extract was injected three times. The RSD obtained for these values were shown to be < 20 % and was acceptable according to the recommendations of Bassett *et al*. (2000).

Data collection

CPF application data collection: Names of the farmers at the research site and the area cultivated by each of them were collected from the Cultivation Officer at Marassana. The type of crop cultivated, the cultivation period and the usage of pesticides were collected from the farmers themselves by meeting on a regular basis. Names of the pesticides applied, amounts of each pesticide applied and the reason for the application were meticulously collected from the farmers on an individual basis.

From the pesticide information collected, CPF (at 40 %) (commercially available formulation, 40 % w/v of CPF) application data were summed up separately in each catchment. The farmers used 10 L of water to dilute 28 mL or more of CPF (at 40 %), and hence the minimum spraying concentration of CPF was found to be 1.12 g/L. It was observed that those who selected CPF for insect control applied CPF at regular time intervals, and these intervals may change according to the crop cultivated on each land.

Rainfall data collection: Rainfall data were collected *in-situ* using a rain gauge prepared according to Wrage *et al.* (1994). The least count of the rain gauge was 1 mm. The total rainfall received at Marassana area during the study period was 350 mm, which is a relatively low value.

Auxiliary data collection: During the interviews with the farmers, information on safety precautions taken during pesticide application, modes of equipment handling, any related health hazards and chronic illnesses experienced by them were gathered. Atmospheric and wet bulb temperatures (^oC) and stream water flow rates (m^3/s) up to three decimal figures (using a current meter, ATOT Pro.No. 03) were measured on a regular basis.

Statistical analysis

CPF concentration in the surface water was assessed by examining the summary statistics of the data collected at the two locations in the stream, L_1 and L_2. Similarly, CPF concentration in the groundwater was assessed by examining the summary statistics of the data collected at the two wells, W_1 and W_2. The eqality of means of CPF concentration at the two locations of surface water as well as at the two wells were tested using analysis of variance (ANOVA).

The pesticide concentration in water has been modelled as a function of a variety of explanatory variables using MLR by several researchers (Törnqvist, 1998; Barbash *et al*., 2001; Muller *et al*., 2002; Kreuger & Sprague *et al*., 2007). A similar procedure was followed in this study and the following MLR model was used:

$$C_i = \beta_0 + \beta_1 R_i + \beta_2 P_i + \varepsilon_i \quad ...(1)$$

where $i=1..N$ (N= sample size), C denotes the CPF concentration (µg/L) in surface water (or in groundwater), R denotes the cumulative rainfall between sampling (mm), P_t denotes the cumulative CPF application between sampling (L), and β_0, β_1 and β_2 are parameters to be estimated. Random error term ε is assumed to be normally distributed with a zero mean and a constant variance σ^2 ($\varepsilon \sim N(0, \sigma^2)$).

Applicability of the above model should be restricted to the peak pesticide application period with usual rainfall pattern, since the data used to estimate the parameters of the model were collected under such conditions. During the peak pesticide application period, it is highly probable that P is non zero for a prolonged period of time.

Uncertainty analysis

In view of the limited number of data used in the statistical analysis, it was highly likely that the estimates of the parameters β_0, β_1 and β_2 of equation 1 be different from the true parameters. Therefore, CPF concentrations predicted using the estimated regression equation would be subjected to uncertainties. Moreover, rainfall and pesticide applications in the field could vary in different situations. Uncertainties in the estimated parameters and the input variables of the model were transformed as a probability distribution of the CPF concentration using a Monte Carlo stochastic simulation procedure (Smith, 2002) as follows:

Step 1: Normal distribution having the estimated mean (µ) and the estimated standard deviation (σ) of a chosen parameter was used to describe the uncertainty in the said parameter, and was denoted by N(µ, σ).

Step 2: Input variables were described by normal distributions having respective mean and standard deviation (σ) tabulated in Table 1.

Step 3: Numerical values of the parameters and variables were generated randomly from the chosen normal distributions. In this study, 10,000 possible values were generated for each parameter.

Step 4: Randomly generated parameters and input variables were used in the model to simulate CPF concentration.

Step 5: The simulated CPF concentration would describe a probability distribution, from which the degree of confidence in the CPF concentration in the surface water (or groundwater) was assessed.

RESULTS AND DISCUSSION

Table 1 provides summary statistics of the CPF concentration data, CPF applications and rainfall. There were 38 observations for each variable listed in Table 1, with minimum values being zeroes in all cases, except in one case where the value is almost close to zero.

In case of surface water contamination, maximum CPF concentrations were recorded as 3.71 µg/L and 2.154 µg/L at L_1 and L_2 respectively. It is of interest that these maximum concentrations were recorded on the same day [22nd of May, see Figure 2 (a)]. The corresponding cumulative rainfall between two consecutive samplings was 70 mm (the second largest rainfall recorded during the study period). The corresponding cumulative CPF applications between samplings were 0.896 L and 0.140 L at catchments A and B, respectively.

In case of groundwater contamination, maximum CPF concentrations were recorded as 7.089 µg/L and 3.250 µg/L at W_1 and W_2 respectively, which were recorded on the same day [18th June, see Figure 2(b)]. The corresponding cumulative CPF application between two consecutive samplings in catchment A was 3.584 L, which was the maximum recorded CPF application (Table 1). It must be noted that there was no rain at the catchments since the 10th of June. It was therefore probable that the CPF applied could not have been washed off into the surface water sources, and may have leached through the soil from irrigated water to the groundwater.

The lower bound of the mean and median of the CPF concentrations tabulated in Table 1 being 0.205 µg/L (corresponding to W_2) clearly demonstrated that the surface water and groundwater were indeed contaminated by CPF during the period of sample collection. Moreover, it could be said with 75 % confidence that CPF concentrations lay in the range bounded by the values corresponding to 12.5 and 87.5 percentiles (tabulated in Table 1). Thus, it could be concluded with 75 % confidence that, during the period of the study, CPF concentration varied in the range 0.111–1.023 µg/L at L_1,

Table 1: Summary statistics of CPF concentrations, CPF applications and rainfall data

Statistic	CPF concentration in water (µg/L)				CPF application in catchments (L)		Rain fall (mm)
	L_1	L_2	W_1	W_2	A	B	
Observations	38	38	38	38	38	38	38
Minimum	0.020	0.000	0.000	0.000	0.000	0.000	0
Maximum	3.710	2.154	7.089	3.250	3.584	1.484	86
Mean	0.588	0.453	0.695	0.565	0.908	0.404	10
Std. dev.	0.668	0.486	1.180	0.783	0.884	0.359	20
Median	0.390	0.212	0.412	0.205	0.560	0.338	0
Sum					34.5	15.4	377
12.5th percentile	0.111	0.095	0.035	0.030	0.000	0.000	0
87.5th percentile	1.023	0.959	1.252	1.170	2.016	0.812	31
25th percentile	0.191	0.124	0.102	0.090	0.224	0.084	0
75th percentile	0.691	0.702	0.723	0.690	1.512	0.616	12

Note: Symbols L_1 and L_2 stand for fresh water sampling locations, W_1 and W_2 for groundwater sampling locations. Symbols A and B represent catchments A and B, respectively.

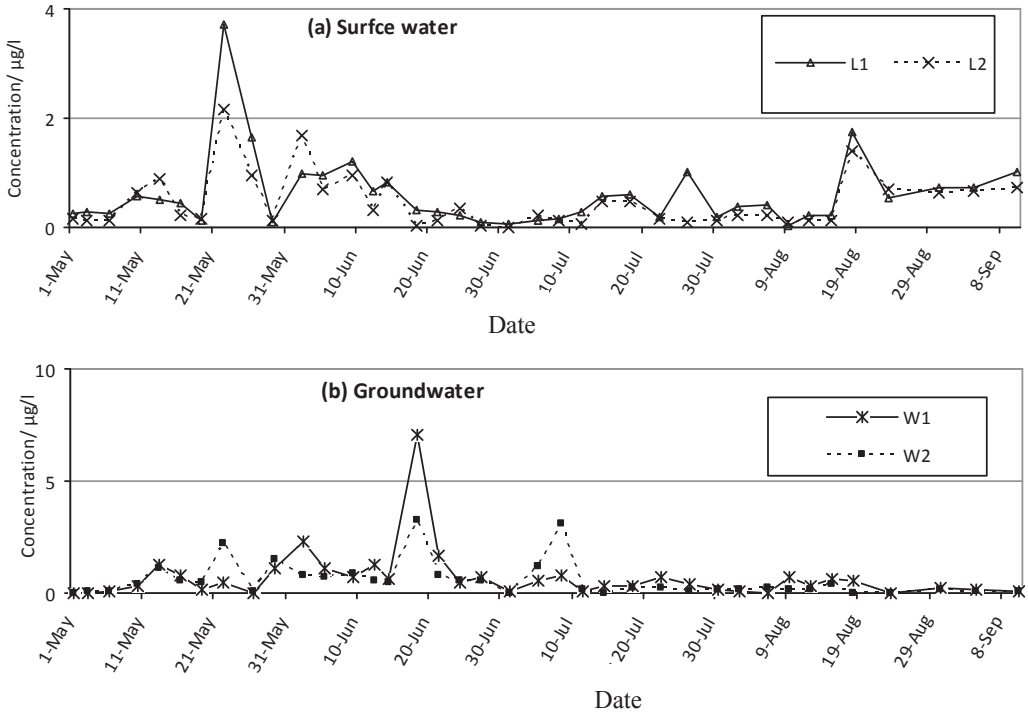

Figure 2: Variations of CPF concentrations (a) surface water and (b) ground water

0.095 – 0.959 µg/L at L_2, 0.035 − 1.252 µg/L at W_1 and 0.030 − 1.170 µg/L at W_2. The respective ranges at 50 % confidence were 0.191 − 0.691, 0.124 – 0.702, 0.102 – 0.723 and 0.090 – 0.690. CPF contamination of the surface water and groundwater during the study period was therefore established.

Summary statistics of CPF application (Table 1) shows that the CPF application in catchment A was nearly as twice as that of catchment B. Total application weight of CPF in catchment A was 14 kg, and in catchment B was 6.5 kg, applied at concentration of 1.12 g/L. Mean and median values of CPF applications provided evidence to the steadiness of CPF applications during the period of study. The maximum cumulative rainfall in the catchments between samplings was recorded as 86 mm, and the total rainfall during the study period was 377 mm. The mean rainfall was 10 mm, modal value of rainfall was zero, and rainfall was below 31 mm even at a 75 % confidence level. All these observations indicate the period of study was a relatively dry one.

Table 2: Test results for equality of means between CPF concentration series

Data series compared	ANOVA F-test statistics	Probability
CPF concentrations at L_1 and L_2	1.01	0.319
CPF concentrations at W_1 and W_2	0.32	0.574

Note: Symbols used are same as in Table 1

Table 2 shows the results of the tests for equality of means between CPF concentration series, obtained using the package StatTools™. In the case of CPF concentrations of surface water and groundwater, ANOVA statistics were 1.01 and 0.32, respectively, and the corresponding probability values were above 0.3. Therefore, there was no statistical evidence to conclude that CPF concentrations differ across the two locations of the surface water (or of the groundwater). Therefore, the CPF concentrations at the two locations of surface water were considered together in the regression analysis reported below. The same procedure was followed with the CPF concentrations at the two locations of groundwater.

CPF in surface water

Regression model: In the tabular data used for estimating the parameters of equation 1, CPF concentrations at L_1 were listed against corresponding cumulative rainfall and CPF application in catchment A. CPF concentrations at L_2 were listed against corresponding cumulative rainfall and CPF application in catchment B. Regression result obtained with StatTools™ is given below:

$$C_s = \underset{[0.0742]}{0.2299^{**}} + \underset{[0.0025]}{0.0213^{****}} R + \underset{[0.0694]}{0.1202} P_t^{*} \quad ...(2)$$

where C_s represents CPF concentration in surface water, standard errors are given within the square brackets below the respective parameters, and symbols ****, ** and * denote p value being less than 0.0001, 0.01 and 0.1, respectively.

Regression statistics corresponding to equation 2 were $R^2 = 0.50$, adjusted $R^2 = 0.49$, standard error of estimate = 0.4184, and F-ratio = 36.6 and probability (F-statistic) < 0.0001, hence the above model could be considered statistically significant in an overall sense. Adjusted R^2 being 0.49 revealed that 49 % of the variability in the CPF concentration in surface water was explained by rainfall and CPF application. It was noteworthy that the multiple R value, the correlation between the actual and fitted CPF concentrations was 71 %. The estimated parameters of equation 2 revealed that 1 mm of cumulative rainfall received in the catchments could cause a 0.021 µg/L increase of CPF concentration in the surface water by holding the other variables constant. The P variable measures the cumulative amount of CPF applications. The results indicate that 1 L of CPF (40 %) applied (400g of CPF) in the catchments (cultivation area) during previous three days could cause 0.120 µg/L increase of CPF concentration in the surface water, when the other variables are held constant, provided CPF applications and rainfall followed similar patterns as the one reported in this study.

As the pesticide application variable in equation 2 was significant only at 90 % confidence and the rainfall variable is highly significant at 99.99 %, this result appears a little uncharacteristic since CPF application is the main variable, which initiates the contamination. P_t is the current cumulative pesticide application (i.e., pesticides applied during previous 2nd day to 4th day). The base model was modified by adding the first lag value of P_t; pesticides applied during previous 5th day to 7thday (P_{t-1}) and second lag value of P_t; pesticides applied during previous 8th day to 10th day (P_{t-2}), while keeping Pt in the model. The comparison of the regression statistics of the base model and modified model are shown in Table 3.

The F-ratio of the modified model is 23 with a probability (F-statistic) < 0.0001; hence the model is statistically significant in an overall sense and therefore the model may be accepted. The R^2 value and the adjusted R^2 value have increased up to 0.57 and 0.55, respectively. Accordingly a 55 % of variability of CPF in surface water is described by the variables included in the model. Schwarz criterion is less than that of the base model as shown in Table 3. The multiple R value, the correlation between actual value and the fitted value of the modified model is 74 %, higher than the said value of the base model.

Table 3: Comparison of regression statistics of the base model and the modified model of surface water

Model parameters	Base model	Modified model
P_t	0.12*	0.055
	(p = 0.08)	(p = 0.42)
P_{t-1}	-	0.098
		(p = 0.14)
P_{t-2}	-	0.183***
		(p = 0.0089)
R	0.021****	0.021****
	(p = 0.0000)	(p = 0.0000)
β_0	0.229***	0.082
	(p = 0.002)	(p = 0.3313)
R^2 value	0.5	0.57
Adjusted R^2 value	0.49	0.55
F-statistic	36.629	23.109
Prob. (F-statistic)	0.00000	0.00000
Schwarz criterion	1.238	1.212
Standard error	0.4184	0.3972

**** p-value< 0.0001 *** p-value< 0.01 * p-value <0.1

The second lag value of cumulative pesticide application (P_{t-2}) is significant at $p < 0.001$. This (P_{t-2}) shows an effect on CPF concentration of surface water at 99 % confidence level, while holding the other parameters constant. The rainfall (R) variable is highly significant at $p < 0.0001$ and shows an effect on CPF concentration of surface water at 99.99 % confidence level when the other variables are held constant. According to the above criteria, it is clear that the modified model provides better prediction of CPF concentration of water in an overall sense.

The modified regression model shows that even when there is no current cumulative application of CPF, the CPF levels at the catchment will not become zero. The rainfall, provides a transport medium to the leftover CPF at the catchment to reach water resources thus becoming the most significant variable explaining the CPF contamination of surface water. Moreover, it is interesting to see that the intercept β_o is significant at 99 % confidence level in the base model and has become insignificant in modified model with a diminished value. This implies that in the base model, β_o might be representing omitted variables in the model. In the modified model, β_o is insignificant and the SIC value is lower, which indicates that it is a better fit than the base model.

It must be noted that the rainfall variable was associated with a p value < 0.0001, which means that the said parameter was statistically significant at 99.99 % level of confidence. The second lag value of CPF application was also significant at 99 % level of confidence. A number of previous studies (Leiguo *et al*., 2004; Ntow *et al.*, 2008) also have established that rainfall and pesticides application has an impact on the pesticides concentration in surface water. This study demonstrated that the quantities of pesticides used in the catchment area and rainfall were the most important estimators of the level and amount of pesticides occurring in the stream. High confidence level of the rainfall parameter was to be expected since rainfall could have a direct impact on CPF concentration in surface water. The following mechanism is suggested: Part of the CPF applied to the vegetable crops could fall directly on the soil surface, and in the absence of rainfall, it would remain in the soil for a period of time as the half-life of CPF is about 10 to 120 days (Singh, 2003). Since the soil at the site was sandy clay loam, having 45 to 80 % sand, and the organic matter content of the soil was as low as 3.0-3.2 % affinity of CPF to soil is poor at the studied site (Chai *et al*., 2009; Menike *et al*., 2011). Therefore, in the event of rainfall, CPF could easily be carried over to the stream, either *via* the runoff or through leaching despite the low solubility for CPF in water. Even in the case of CPF adsorbed in soil, presence of water after a rainfall could cause desorption of CPF, and the runoffs may take CPF to the nearest surface water source. Further, the soil itself may be carried to the surface water sources by the surface runoffs at high rainfall intensities.

CPF application variable in the 2 models described above were statistically significant only at a low level of confidence compared to rainfall. This is to be expected since CPF applied on the field needed a carrier, such as rainfall, to take the CPF to the surface water. The period of study was relatively dry and the results of MLR are acceptable according to the CPF transport mechanism described above.

Uncertainty analysis: Even though the regression model given by equation 2 could be considered reasonable in describing the interrelationship among CPF concentration in the surface water, CPF application and rainfall, it was probable that the estimated parameters were different from the true parameters. Moreover, the input parameters could also be considered to possess uncertainty. Thus the said model was subjected to the uncertainty analysis outlined previously.

Equation 2 provided the mean and the standard deviation of the rainfall parameter as 0.0213 and 0.0025, respectively, and those of the CPF application parameter as 0.1202 and 0.0694, respectively. The mean and standard deviation of the intercept were given by 0.2299 and 0.0742, respectively. In the uncertainty analysis carried out, rainfall parameter, CPF application parameter and the intercept were therefore considered being described by the random distributions N(0.0213, 0.0025), N(0.1202, 0.0694) and N(0.2299, 0.0742), respectively. The input data on rainfall and CPF applications were described by the random distributions N(10, 20) and N(0.6559, 0.7164), respectively.

Using the said normal distributions, 10,000 possible values were randomly generated for each parameter, intercept and input variables, which were then used in the linear model (similar to equation 2) to simulate CPF concentrations. Results of the Monte Carlo stochastic simulation obtained using @RISK are shown in Figure 3.

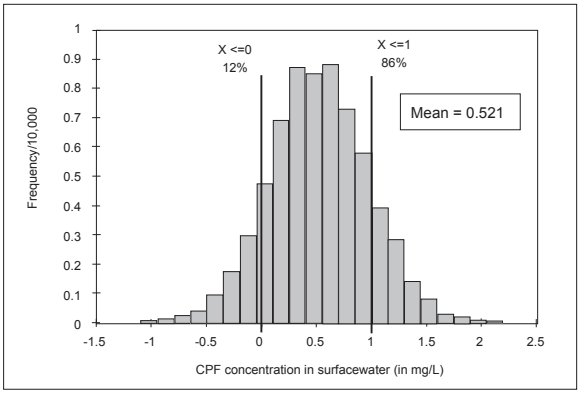

Figure 3: Probability distribution of the simulated CPF concentration in the surface water

The left most delimiter (or grey vertical line overlaying the histogram) was at the 12th percentile (marked by 12 %) having the *x* value less than or equal to zero. This means that there is a 12 % probability that the CPF concentration in the surface water was zero (since it cannot be negative). The rightmost delimiter was at the 86th percentile (marked by 86 %) having the *x* value less than or equal to unity, hence there was a 14 % probability that the CPF concentration in the surface water was above 1 µg/L. Therefore, the probability of CPF concentration of surface water lying between 0 and 1 µg/L was 74 %.

The analysis carried out above with @RISK showed that there was a 50 % probability for CPF concentration in the surface water to be between 0.221 and 0.822 µg/L, with the mean CPF concentration being 0.522 µg/L. Therefore, the mean value of CPF concentration of surface water did not exceed the maximum permissible level of (0.55 µg/L) total pesticides (NER, 2008) at a 50 % probability. The mean value of CPF concentration of surface water was higher than the maximum permissible level (0.041 µg/L) for CPF in fresh water according to the USEPA (2009) water quality criteria.

CPF in groundwater

Regression model: Since the results of Table 2 showed that CPF concentrations in the groundwater did not have a statistically significant difference across the two locations, they were considered together in estimating the parameters in equation 1. The rainfall remained the same for the two locations. Since both wells were situated in catchment A (see Figure 1), CPF application data also remained the same as that of catchment A. Regression result obtained with StatTools™ is given below:

$$C_g = \underset{[0.1567]}{0.0226} + \underset{[0.0050]}{0.0016}\,R + \underset{[0.1130]}{0.6508}\,P_A^{****} \quad ...(3)$$

where C_g represents CPF concentration in groundwater, subscript $_A$ represents catchment A, standard errors are given within the square brackets below the respective parameters, and symbol **** denotes p value being less than 0.0001.

Regression statistics corresponding to equation 3 were $R^2 = 0.32$, adjusted $R^2 = 0.30$, standard error of estimate = 0.8330, and *F*-ratio = 17.2 with p value < 0.0001. Even though the overall model was statistically significant, judging by the p value of *F*-ratio, rainfall parameter and intercept were not statistically significant. CPF application parameter, on the other hand, was statistically significant at 99 % level of confidence. Explanation of this observation may be that in the presence of irrigated water, CPF residues on the soil surface are carried downwards through soil (Huggenberger *et al*., 1973). In the event of such continuous movement, it is probable for CPF to reach the water table (which is never below 1.5 m depth close to the wells) and contaminate groundwater. However, since adjusted R^2 was as low as 0.30, only 30 % of the variability in the CPF concentration in groundwater could be explained by CPF application. Correlation between the actual and fitted CPF concentrations being 57 % pointed to the fact that equation 3 could only poorly describe the CPF concentrations in groundwater. Therefore, equation 3 was abandoned as a model incapable of explaining CPF concentration in groundwater.

CPF mobility in soil: As indicated in the preceding section, CPF contamination of groundwater may be explained by CPF applied in the field being transported through the soil to groundwater. In order to test the above hypothesis, the selected soil characteristics and the downward movement of CPF through soil at Site D (shown in Figure1) has been analysed up to 60 cm depth in soil, at 15 cm intervals (Menike *et al*., 2011). They demonstrated that CPF moves downward through soil up to 60 cm depth in about two days. According to the physical and chemical properties reported, the soil in the study area was acidic and the major part of the soil was identified as sand. The soil in the area being seriously deprived of organic fertilizers such as compost and/or manure, the organic matter content was low. These properties of the soil account for the low value (1.875 L/kg) of the sorption coefficient *(K_d)* of CPF in soil (Menike *et al*., 2011). Thus it was concluded that the above mentioned coherent features support the weak adsorption affinity of CPF to soil and may cause CPF to move downwards through the soil with relative ease, resulting in groundwater contamination.

Since the pesticide application data were collected once in three days, regression analysis reported in the preceding section was repeated with the current and previous pesticide application data. The result obtained using StatTools™ is given below:

$$C_{gt} = \underset{[0.2059]}{-0.1207} + \underset{[0.0049]}{0.0019} R_t + \underset{[0.1149]}{0.6378} P_{At}^{****} + \underset{[0.1122]}{0.2208} P_{A,t-1}^{**} - \underset{[0.1128]}{0.0333} P_{A,t-2} \quad ...(4)$$

where C_{gt} represents CPF concentration in groundwater, subscript $_A$ represents catchment A, standard errors are given within the square brackets below the respective parameters, and symbol **** denotes p value being less than 0.0001.

Where P_{At}, current cumulative pesticides application was the pesticides applied during the three days (previous 2nd day to 4th day) prior to sampling and $P_{A, t-1}$ and $P_{A, t-2}$ are first lag value and second lag value of $P_{At,}$ as explained earlier in this paper.

R^2, adjusted R^2, standard error of estimate and *F*-ratio values were 0.37, 0.33, 0.8237, and 12.9, respectively with p value < 0.0001. The correlation between the actual CPF concentration and the predicted CPF concentration was also increased to 60 %.

The parameters and statistics of equation 4 were the same as equation 3, with the parameter of P_A being highly significant as in equation 3. The added characteristic in equation 4 was that the coefficient of the first lag value of P_A and this was also statistically significant at 95 % level of confidence, whereas the second lag value was not statistically significant. This implies that CPF applied to the field within the last three days and six days can have a statistically significant effect on the C_g , but the latter is not affected by CPF applied nine days prior to sampling. These results corroborated the results of CPF mobility studies reported above.

CONCLUSION

CPF was a widely used insecticide in the selected commercial grade vegetable cultivation area in Marassana. One of the objectives of this study was to estimate the CPF contamination of surface water and groundwater in the said area. Throughout the vegetable cultivation period from May - September, the selected surface water resource, Kiwullinda Oya stream and ground water resources were found to be positive for CPF, demonstrating that the surface water and groundwater were indeed contaminated with CPF.

It is concluded that the CPF concentration across the two locations was not statistically significantly different. The proposed linear regression model was statistically accepted and demonstrated that the CPF concentration in the stream water varied with rainfall (99 % confidence limits) and with CPF application (90 % confidence limits). These findings established that the CPF applied on the field needed a carrier, such as rainfall, to take the CPF in to the surface water. This occurrence could be explained in the following way; when the CPF application is heavy, low adsorption affinity to soil leads to lingering free CPF within the soil that can be easily carried into the stream either *via* runoff or through leaching despite the low solubility of CPF in water. CPF may also enter the stream with soil that is carried by surface runoff at high rainfall intensities.

A 1 mm of cumulative rainfall received in the catchments result in an increase of CPF concentration in surface water by 0.021 µg/L when the CPF application variable is held constant, and a 1 L of CPF (40 %) applied in the catchments cause an increase of CPF concentration in the surface water by 0.120 µg/L when the rainfall variable is held constant, provided similar geographical and climatic factors as the one reported in this study prevails.

The probable uncertainty between the estimated parameters and true parameters was estimated using a suitable tool, the Monte Carlo stochastic simulation. The

results of the simulation show an 88 % probability that the CPF concentration in the surface water is more than 0 µg/L. It also shows that there was a 14 % probability that the CPF concentration in the surface water is above 1 µg/L. The probability of CPF concentration of stream water exceeding the maximum permissible level for total pesticides in freshwater (NER, 2008) was less than 50 %. The mean value was fairly larger than the maximum permissible level (0.041 µg/L) for CPF in fresh water according to the USEPA (2009) water quality criteria.

CPF is transported through soil within a maximum of 6 days into ground water, as a result of low organic matter content and the sandy base soil structure. These results corroborated the results of regression analysis carried out with current and previous pesticide application data. The current cumulative application of CPF was highly significant at 99 % confidence level and the first lag value of the current cumulative pesticides application (which included the CPF applied from previous 4th day to 7th day) was significant at 95 % confidence level demonstrating the effect of these two factors on the CPF concentration of ground water.

Results of this study demonstrated that for a given watershed, rainfall and CPF use are the two major factors controlling the dynamics of CPF transport into surface water, whereas CPF application, the soil structure, and soil organic matter content are the major factors controlling CPF transport into groundwater.

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Do Targeted Bans of Insecticides to Prevent Deaths from Self-Poisoning Result in Reduced Agricultural Output?

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Background: The pesticides monocrotophos, methamidophos, and endosulfan were a very common cause of severe poisoning in Sri Lanka during the 1980s and early 1990s, before they were banned in 1995 and 1998. Now, the most commonly used insecticides are the less toxic World Health Organization Class II organophosphorus pesticides and carbamates. These bans were followed by a large reduction in both fatal poisonings and suicide in Sri Lanka.

Objective: We aimed to see if these bans adversely affected agricultural production or costs.

Methods: We used data from the World Resources Institute to compare the yields of the main crop groups in Sri Lanka with those from surrounding South Asian countries for 1980–2005. We also examined data from the Sri Lankan Department of Census and Statistics to examine the yields of 13 specific vegetable crops and rice for 1990–2003, along with the costs of rice production.

Results: We found no drop in productivity in the years after the main bans were instituted (1995, 1998). We observed substantial annual fluctuation in estimated yields in all data sources, but these did not coincide with the bans and were no larger than the fluctuations in other countries. Also, there was no sudden change in costs of rice production coinciding with bans.

Conclusions: Countries aiming to apply restrictions to reduce deaths from pesticide poisoning should evaluate agricultural needs and develop a plan that encourages substitution of less toxic pesticides. If farmers have an affordable alternative for pest control for each crop, there is no obvious adverse effect on agricultural output.

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Pesticide self-poisoning is a major problem in rural areas of the Asian Pacific developing world (Eddleston and Phillips 2004; Konradsen et al. 2005). Widespread agricultural use of pesticides and home storage make them easily available for acts of self-harm in many rural households. Some clinicians have called for bans of particular pesticides that cause major local problems (Daisley and Hutchinson 1998; Siwach and Gupta 1995). Others within both the clinical and agricultural communities have called for the removal of all highly toxic pesticides on public health grounds [Eddleston et al. 2002; Food and Agriculture Organization of the United Nations (FAO) Committee on Agriculture 2007; Konradsen et al. 2003]. However, some agronomists and the pesticide industry have warned that such bans may adversely affect agricultural output or prices in the affected regions (Cooper and Dobson 2007; Oerke and Dehne 2004; Oerke et al. 1994). Thus far, however, there have been no studies to support or refute these assertions.

The problem of pesticide self-poisoning in the context of high suicide rates has been recognized at the highest levels in Sri Lanka. The World Health Organization (WHO) Class I toxicity organophosphorus pesticides (OP) were a very common means of suicide in the 1980s and early 1990s (Roberts et al. 2003; Van der Hoek and Konradsen 2006). Parathion and methylparathion were banned in the mid-1980s, and the last of the Class I OPs, monocrotophos and methamidophos, were banned in 1995 (Roberts et al. 2003; Van der Hoek and Konradsen 2006). Unfortunately, their place in the market was taken by endosulfan, resulting in an epidemic of self-poisoned patients with status epilepticus and many deaths (Roberts et al. 2003). This insecticide in turn was banned in 1998, and the current most commonly used insecticides are the WHO Class II OPs and carbamates (Roberts et al. 2003; Van der Hoek and Konradsen 2006).

In a previous study (Roberts et al. 2003) carried out in the hospitals of the North Central Province (NCP) of Sri Lanka, we showed that these bans resulted in these three pesticides being no longer the cause of any poisonings within a few years. Although the number of pesticide poisonings presenting to the hospital did not decrease, there was a significant reduction in the total number of poisoning deaths. We have also shown there has been a 40–50% progressive reduction in suicide by self-poisoning with pesticides and in the overall suicide rate over 1995–2002; these two bans are the most plausible explanation (Figure 1) (Gunnell et al. 2007).

These national public health pesticide bans offer the opportunity to observe the effects of such bans on agricultural output. We therefore investigated whether changes in production and costs for major crops have occurred during the period of these bans; other countries in South Asia have similar problems with very high rates of suicide due to insecticides, but they have not instituted bans. We also wished to compare longitudinal trends in Sri Lankan agricultural production with that in the neighboring countries.

Methods

Sources of data. We obtained longitudinal whole-country data on agricultural production and yields for the South Asian countries of Sri Lanka, India, Pakistan, and Bangladesh from the World Resources Institute (2007) website. These data are collated from data supplied by these countries to the FAO.

Longitudinal data on a range of specific crops for the whole of Sri Lanka was obtained from Sri Lanka's Department of Census and Statistics (2007a). These data are based on regular surveys conducted by the Ministry of Agriculture.

Paddy rice is the most important agricultural product in Sri Lanka (International Rice Research Institute 2007). To further explore the range of possible factors involved in altering production and costs, we obtained longitudinal data for the NCP on paddy productivity,

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costs, and consumer price index movements. These came from the Department of Census and Statistics (2007) and the Ministry of Agriculture database through the Hector Kobbekaduwa Agrarian Research and Training Institute (Colombo, Sri Lanka). The Ministry of Agriculture estimates are based on annual field surveys.

Results

The yields of the main crop groups in Sri Lanka showed no obvious drop in productivity in the years after the main bans were instituted (1995 and 1998). There was substantial annual fluctuation in estimated yields, but these did not coincide with the bans and were no larger than the fluctuations in other countries. On average, the Sri Lankan yields for cereals and pulses are higher and those for roots and tubers are lower than those of the neighboring countries (Figure 2).

The data on the Sri Lankan yields of 13 vegetable crops during 1990–2003 also show no obvious drops in productivity at the time of the bans (Figure 3). We did observe some downward trends in cucurbit (pumpkins, cucumber, and gourds) vegetable production that predate these bans, but otherwise, production yields have been remarkably constant, with the obvious seasonal variation due to the different monsoonal rainfall with the Maha and Yala seasons. We examined the production of tea, rubber, and coconut, and these also did not change during this time (data not shown).

Rice paddy production varied with the Maha and Yala seasons, but we saw no other apparent change at the time of the bans (Figure 4). Production costs for paddies within the NCP have increased steadily over time. However, there was no change in this rate that coincided with the bans; in contrast, a significant increase in production costs occurred because of rising fuel prices and deterioration of the exchange rate around 2002–2003. We examined the production costs of a number of other crops (data not shown), none of which showed a change in the trend at this time.

Discussion

In the present study, we found no good evidence that a pesticide ban necessarily results in reduced output or increased costs to the farmer. Overall, we found no significant change in food production during the 1990s, and no change in the rate of increase in production costs or yield that could be attributed to the pesticide restrictions.

The pesticides were targeted on the basis of Ministry of Health data indicating that specific insecticides were of concern because of large numbers of poisoning deaths. However, the bans of specific insecticides were coordinated by the Ministry of Agriculture. Therefore, the needs of farmers to have an affordable insecticide for pest control for each crop were taken into account. Before the regulations, in 1988–1990, monocrotophos and methamidaphos were widely used. They accounted for 60–75% of the total volume of OPs imported each year (Ministry of Agriculture, unpublished data). These two OPs were also approved for use on a wide variety of crops, and yet their bans led to no obvious adverse effect on agricultural output of any single crop. For each crop and pest, a number of other affordable pesticides with equivalent activity were approved and available for use.

Rice is the most important crop in Sri Lanka (International Rice Research Institute 2007). Our results are consistent with findings of integrated pest management (IPM) programs, indicating that the need for insecticides in rice is often overestimated. These programs in Sri Lanka have lead to large reductions in total pesticide usage and increased yields (Van den Berg et al. 2002), and similarly favorable impacts of IPM have been found for many other crops in other countries (Van den Berg 2004). Studies of rice yield in Sri Lanka have identified that production is largely determined by water supply, nutrient content of the soil, and cultivar. Losses due to pests are not regarded as an important determinant of yield in recent decades after the green revolution because of improved cultivars and the use of pesticides (Dhanapala 2007). However, to investigate minor changes in production or costs related to pesticide regulation would require prospective agricultural studies that carefully control for these other factors that together largely determine rice yields (e.g., a study performed by a national rice research center).

The problem of pesticide poisoning is widespread within the region. Other countries aiming to apply pesticide restrictions to reduce poisoning incidents and deaths should bear in mind the needs of agriculture in order to be accepted and receive cooperation from the local communities. An increase in production costs might have occurred if there had been enforced use of more expensive or less-efficient pesticides. However, in the present study, the only correlation was with worsening foreign exchange rates that made imports more expensive, particularly fuel prices. The 2.5-fold increase in production costs for paddies is in line with the 2.5-fold deterioration in the exchange rate and the 3.5-fold increase in the

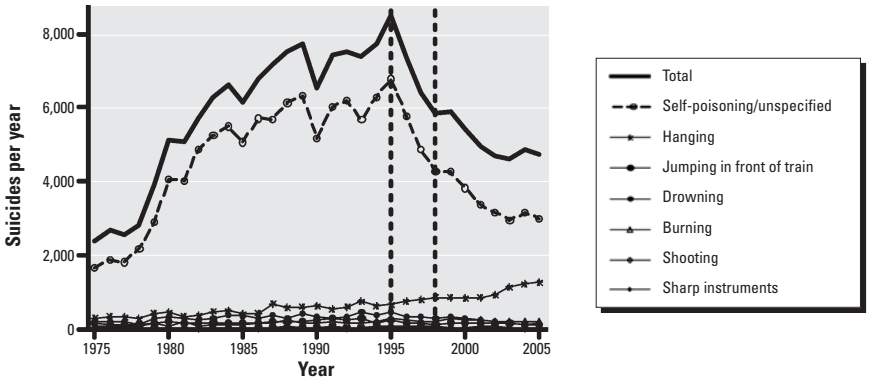

Figure 1. Change in suicide rate and deaths from poisoning between 1975 and 2005. Vertical lines indicate the years when all Class I insecticides (1995) and endosulfan (1998) were banned. Adapted from Gunnell et al. (2007).

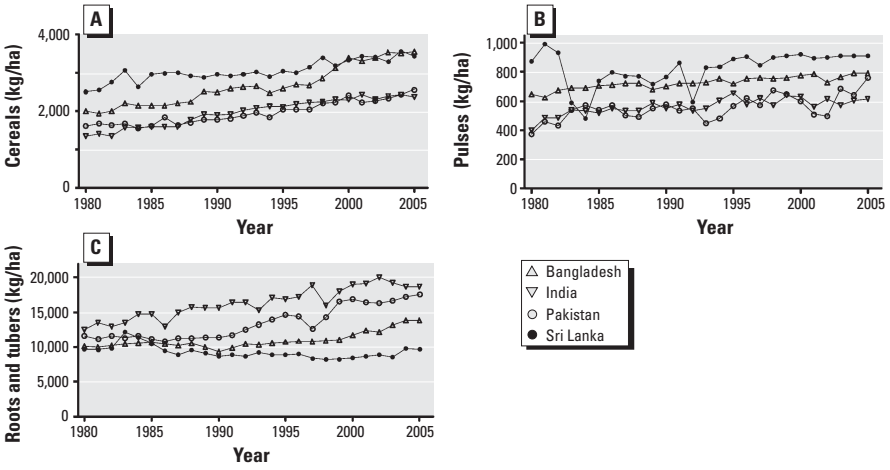

Figure 2. Yields of cereals (*A*), pulses (*B*), and roots and tubers (*C*) in south Asian countries during 1980–2005.

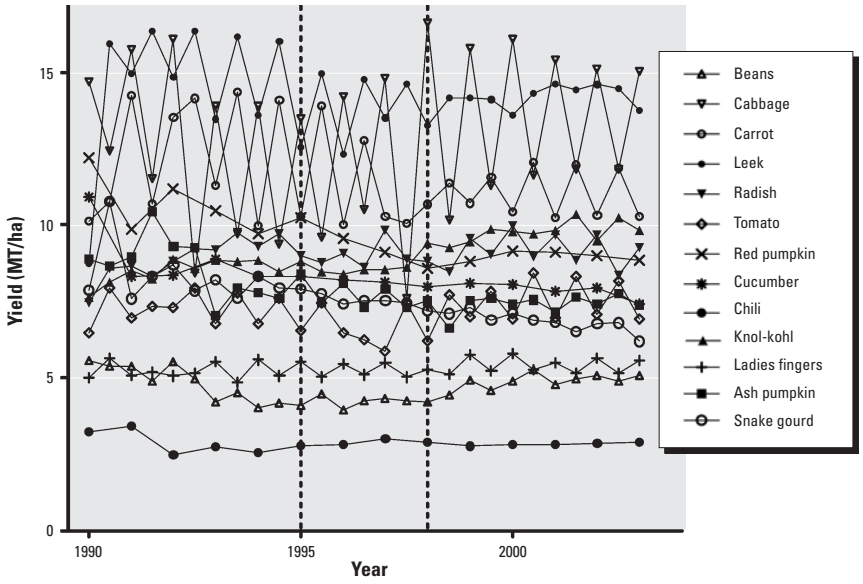

Figure 3. Longitudinal changes in Sri Lankan yields of 13 vegetable crops during 1990–2004. MT, metric tons. Vertical lines indicate the years when all Class I insecticides (1995) and endosulfan (1998) were banned.

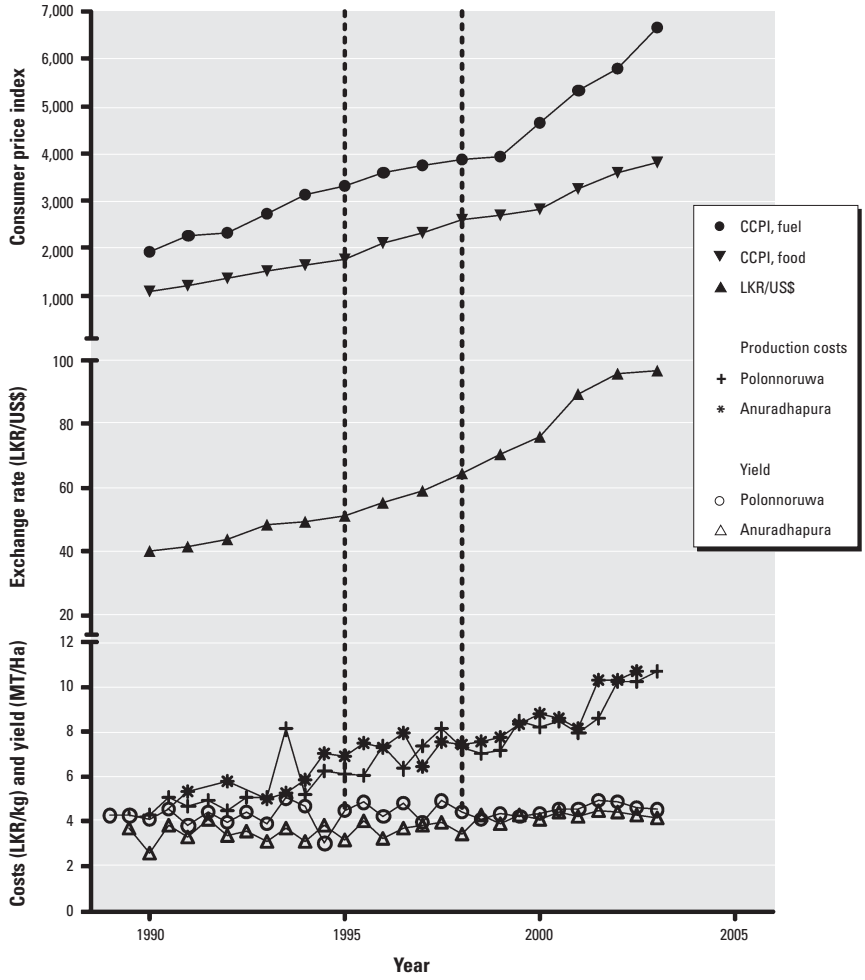

Figure 4. Longitudinal changes in paddy production and costs of production in two districts (Anuradhapura and Polonnaruwa) of the NCP and correlation with external major cost items. Abbreviations: CCPI, Colombo Consumer Price Index; LKR, Lanka rupees; MT, metric tons. Vertical lines indicate the years when all Class I insecticides (1995) and endosulfan (1998) were banned.

Sri Lankan cost of living index during 1990–2003. Again, this is not surprising because the price of the banned pesticides was not less than that of comparable OPs in this case. Moreover, agrochemicals such as fertilizer and pesticides are currently a relatively small component of the total costs of rice production compared with labor and fuel, which together account for > 75% of total production costs (International Rice Research Institute 2007). However, regulatory restrictions to shift agricultural use to newer, less toxic non-anticholinesterase insecticides might lead to significant price increases. Studies are now being undertaken to examine the economics and impact of this strategy. There may be additional health benefits from reduced acute and chronic occupational poisoning and therefore other economic benefits because of greater productivity; however, these benefits are far more difficult to quantify.

The initial pesticide bans were largely based on a simple strategy of phased removal of all Class I (extremely hazardous) pesticides in Sri Lanka. This is similar to the global strategy now proposed by the FAO Committee on Agriculture (2007). However, the use of public health data was critical in identifying the specific Class II (moderately hazardous) pesticide that was of more concern (endosulfan). We have recently identified two other Class II OPs with relative greater human toxicity (Eddleston et al. 2005). It will be important to evaluate agricultural needs and a strategy for substitution in developing a regulatory strategy to further reduce deaths.

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Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study

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Summary

Background Although more than 100 organophosphorus insecticides exist, organophosphorus poisoning is usually regarded as a single entity, distinguished only by the compound's lethal dose in animals. We aimed to determine whether the three most common organophosphorus insecticides used for self-poisoning in Sri Lanka differ in the clinical features and severity of poisoning they cause.

Methods We prospectively studied 802 patients with chlorpyrifos, dimethoate, or fenthion self-poisoning admitted to three hospitals. Blood cholinesterase activity and insecticide concentration were measured to determine the compound and the patients' response to insecticide and therapy. We recorded clinical outcomes for each patient.

Findings Compared with chlorpyrifos (35 of 439, 8·0%), the proportion dying was significantly higher with dimethoate (61 of 264, 23·1%, odds ratio [OR] 3·5, 95% CI 2·2–5·4) or fenthion (16 of 99, 16·2%, OR 2·2, 1·2–4·2), as was the proportion requiring endotracheal intubation (66 of 439 for chlorpyrifos, 15·0%; 93 of 264 for dimethoate, 35·2%, OR 3·1, 2·1–4·4; 31 of 99 for fenthion, 31·3%, 2·6, 1·6–4·2). Dimethoate-poisoned patients died sooner than those ingesting other pesticides and often from hypotensive shock. Fenthion poisoning initially caused few symptoms but many patients subsequently required intubation. Acetylcholinesterase inhibited by fenthion or dimethoate responded poorly to pralidoxime treatment compared with chlorpyrifos-inhibited acetylcholinesterase.

Interpretation Organophosphorus insecticide poisoning is not a single entity, with substantial variability in clinical course, response to oximes, and outcome. Animal toxicity does not predict human toxicity since, although chlorpyrifos is generally the most toxic in rats, it is least toxic in people. Each organophosphorus insecticide should be considered as an individual poison and, consequently, patients might benefit from management protocols developed for particular organophosphorus insecticides.

Introduction

Organophosphorus insecticide self-poisoning is a major global health problem,[1,2] with hundreds of thousands of deaths each year.[3,4] Although most such deaths are in the developing world,[4] this poisoning is also an important cause of fatal self-poisoning in developed countries.[5] Organophosphorus insecticides inhibit acetylcholinesterase and butyrylcholinesterase enzymes resulting in overstimulation at cholinergic synapses.[6] Management of severe poisoning is difficult, requiring intensive care and use of atropine and oxime cholinesterase reactivators.[6,7] Management is complicated by the paucity of clinical trial evidence to guide treatment, with no clear evidence for benefit from any therapy other than oxygen, atropine, and diazepam.[8]

Although differences in human toxicity between organophosphorus insecticides were reported in 1977,[9] acute organophosphorus poisoning is regarded as a homogeneous entity in most textbooks and review or research articles. Specific treatment advice for particular organophosphorus insecticides is not supplied,[10] despite wide variation in animal toxicity, fat solubility, metabolism, selectivity for acetylcholinesterase over other serine esterases, side groups attached to the phosphate, and speed of ageing (loss of an alkyl side chain that prevents reactivation by oximes),[11] that might affect poisoning severity and response to treatment.[6,8,12] The system used most widely for differentiating organophosphorus insecticides is a WHO method based on toxic effects in rats after oral dosing.[13] This scheme was developed for occupational poisoning but has been used to ban pesticides that frequently cause death from self-poisoning[14] and to identify highly toxic pesticides.[10]

In this observational study, we aimed to determine whether the three most common organophosphorus insecticides used for self-poisoning in Sri Lanka differ in the clinical features and severity of poisoning they cause. Specifically, we aimed to compare the odds of death, intubation, and seizures, the mode of death, and the response to treatment in patients poisoned by chlorpyrifos, dimethoate, and fenthion. We also examined whether the WHO classification system accurately predicts toxicity in people.

Methods

Patients

Patients were identified at admission to three Sri Lankan hospitals as part of a cohort study of acute self-poisoning that started Mar 31, 2002, in Anuradhapura, June 4, 2002, in Polonnaruwa, and Nov 23, 2002, in Kurunegala. Patients were identified until Feb 19, 2003, in Kurunegala and May 25, 2004, in Anuradhapura and

Polonnaruwa. Patients were included in this study if they had a history of chlorpyrifos, dimethoate, or fenthion ingestion as indicated by the patient or relatives, the transferring doctor, or the pesticide bottle. Patients who ingested more than one organophosphorus insecticide or other poisons in addition to the insecticide (except for alcohol) were excluded from the study.

Patients remained under the care of the hospitals' consultant physicians who had primary responsibility for their management. Management protocols were agreed between the medical team and study team. Decisions about intubation and transfer of patients to intensive care were made by the medical team independently of study doctors. All decisions were based on the patient's clinical condition and not the particular organophosphorus insecticide ingested, as per usual hospital practice. Atropine was given according to a standard protocol.[15] Symptomatic patients received pralidoxime chloride (1 g bolus) followed by further bolus doses of 1 g every 6 h for 1–3 days. Once resuscitated, patients or their relatives were approached regarding recruitment to a randomised controlled trial of activated charcoal that was nested into the cohort; written informed consent was obtained from patients or relatives.

All patients were seen regularly by study doctors at least every 3 h or more frequently, according to clinical need, to check for changes in clinical condition, and to review atropine requirements. Important events, such as endotracheal intubation, seizures, or death were recorded at the time of the event. Patients were also seen on a study ward round twice each day and their condition over the previous 12 h recorded. Patients were first managed on the medical ward. Each hospital had two to eight such beds for medical patients. Seriously ill patients, as judged by the ward's medical staff, were transferred to the intensive care unit as beds became available.

Criteria for intubation were: tidal volume less than 180 mL per breath with a Wright's respirometer; respiratory rate less than ten breaths per min; or failure of a Guedel airway to preserve airway patency. Arterial blood gases were not available to guide therapy. Hypotensive patients (systolic blood pressure <80 mm Hg), who were not responding to 50–100 mg of atropine and fluid resuscitation (with 2 L of normal saline), were treated with dopamine plus dobutamine (both started at 5–10 $\mu g\ kg^{-1}\ min^{-1}$ and increased as necessary) by infusion pump. Norepinephrine and epinephrine infusions were not used; bolus epinephrine (1–3 mg intravenously) was administered for cardiac arrests as per standard Advanced Life Support guidelines. Ethics approval was obtained from Oxfordshire Clinical Research Ethics Committee and Faculty of Medicine Ethics Committee, Colombo.

Procedures

Blood samples were taken from all patients recruited to the randomised controlled trial until December, 2003, and used to test the accuracy of the history of the organophosphorus insecticide ingested for the cohort. Admission plasma samples (taken a median of 3–4 h after ingestion for all three insecticides) were assayed for butyrylcholinesterase activity (to show exposure) and insecticide concentration in 433 patients (240 chlorpyrifos, 136 dimethoate, 57 fenthion).

Red cell acetylcholinesterase activity was assayed in samples taken from 90 consecutive patients in the trial (57 with chlorpyrifos, dimethoate, or fenthion) during two periods (May 9 to July 10, 2002, and Dec 2 to Dec 26, 2002). Lab assay capacity limited the sample number that could be handled and determined the short period of sampling.

For acetylcholinesterase measurement, 0·2 mL of EDTA blood was diluted at the bedside into 4 mL of cooled saline and frozen to –20ºC. Plasma was separated from a second EDTA blood sample and frozen at –20ºC. All analyses were done in Munich. Acetylcholinesterase activity was assayed according to a modified Ellman method.[16] Reactivatability of acetylcholinesterase (its ability to be reactivated by supratherapeutic concentrations of oxime, showing the proportion that is not aged and therefore still potentially responsive to oximes) and butyrylcholinesterase activity were assessed as described.[11,16] Concentrations of organophosphorus insecticides in plasma were quantified by reversed phase high-performance liquid chromatography and ultraviolet detection. The lower limits of quantitation were 1 μmol/L plasma for dimethoate and 0·1 μmol/L plasma for chlorpyrifos and fenthion.

Statistical analysis

We did primary data analysis in SPSS (release 11) and Stata (release 8) software. Demographic factors and clinical characteristics were summarised with counts for categorical variables and the median (IQR) for non-normally distributed continuous variables. We

	Chlorpyrifos (n=439)	Dimethoate (n=264)	Fenthion (n=99)
Demographic characteristics			
Male	340 (77·4%)	193 (73·1%)	63 (63·6%)
Age (years)*	30 (23–40)	30 (22–42)	30 (22–38)
Time to presentation (h)*	4 (2–5)	3 (2–5)	4 (2–7)
Randomised into trial	358 (81·5%)	213 (80·7%)	82 (82·8%)
Activated charcoal treatment			
None	146 (33·3%)	98 (37·1%)	33 (33·3%)
Single dose	153 (34·9%)	87 (33·0%)	35 (35·4%)
Multiple doses	140 (31·9%)	79 (29·9%)	31 (31·3%)
Admission characteristics			
Glasgow Coma Score*	15 (14–15)	14 (6–15)	15 (15–15)
Butyrylcholinesterase activity (mU/mL)*	34 (0–304)	1129 (532–1720)	0 (0–33)
Organophosphorus insecticide plasma concentration (μmol/L)*	1·3 (0·4–3·5)	355·5 (160·0–674·0)	4·9 (0·6–16·6)

Data are number (%) unless otherwise indicated. *Median (IQR). Time of ingestion was known for 428, 259, and 95 patients, respectively. Butyrylcholinesterase and pesticide were measured in 240 and 230 chlorpyrifos patients, 136 dimethoate patients, and 57 and 51 fenthion patients, respectively.

Table 1: **Baseline characteristics following organophosphorus insecticide self-poisoning**

	Chlorpyrifos (n=439)	Dimethoate (n=264)	Fenthion (n=99)
Outcomes			
Number of deaths	35	61	16
Case fatality ratio (95% CI)	8·0% (5·8–10·9)	23·1% (18·4–28·6)	16·2% (10·2–24·7)
Number requiring intubation	66	93	31
Proportion (95% CI)	15·0% (12·0–18·7)	35·2% (29·7–41·2)	31·3% (23·0–41·0)
Number with seizures	8	0	5
Proportion (95% CI)	1·8% (0·9–3·6)	0·0% (0·0–1·4)	5·1% (2·2–11·3)
Admission characteristics for fatal cases			
Glasgow Coma Score	6 (3–14)	3 (3–7)	12 (8–15)
Butyrylcholinesterase activity* (mU/mL)	6 (0–94)	735 (269–1240)	0 (0–941)
Organophosphorus insecticide plasma concentration (μmol/L)	4·7 (3·6–5·9)	846 (657–1183)	12·3 (0·94–30·3)
Acetylcholinesterase activity over time			
Number	18	10	4
On admission (mU/μmol Hb)	63·5 (27·0–124·6)	69·0 (22·1–145·7)	64·2 (32·5–75·4)
After 1 h (mU/μmol Hb)	391·8 (294·8–507·8)	110·2 (59·4–166·9)	68·1 (53·8–122·0)
After 12 h (mU/μmol Hb)	312·5 (205·9–480·2)	42·5 (13·7–67·9)	41·9 (14·6–100·6)
Aged acetylcholinesterase on admission	19·4% (6·4–26·1)	71·9% (57·2–86·8)	70·3% (65·6–82·2)
Aged acetylcholinesterase after 12 h	19·8% (3·5–26·2)	84·5% (80·5–96·0)	85·7% (75·4–91·1)

Data are median (IQR) unless otherwise indicated. *Admission butyrylcholinesterase activity was available for 11, 25, and nine patients with fatal outcome, and organophosphorus insecticde concentration for 11, 25, and eight patients with fatal outcome, poisoned by chlorpyrifos, dimethoate, and fenthion, respectively. Butyrylcholinesterase and organophosphorus insecticide was measured in all patients who were recruited to the trial until December 31, 2003.

Table 2: **Outcomes after admission (plus admission characteristics for fatalities)**

calculated case fatality (and need for intubation) plus 95% CI in the dimethoate and fenthion groups using the Wilson method, CIA software (version 2.0),[17] and compared with chlorpyrifos by calculating odds ratios plus 95% CI. We used logistic regression models to investigate the effects of age, sex, trial recruitment, and charcoal administration on mortality and intubation.

Role of the funding source

The study sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 31, 2002, and May 25, 2004, 5585 poisoned patients were reviewed on admission to the adult medical wards. 1193 (21·4%) had a history of organophosphorus insecticide self-poisoning. All were approached for recruitment to a randomised controlled trial of activated charcoal; 937 (78·5%) were recruited. This trial was stopped in October, 2004, after the planned final interim analysis identified no effect of activated charcoal on death.[18]

About two-thirds of patients poisoned by an organophosphorus insecticide (802 of 1193) reported ingesting one of three pesticides: chlorpyrifos, dimethoate, and fenthion (table 1). The groups were similar at baseline (table 1). 147 patients (12·3%) ingested unknown cholinesterase inhibitors whereas 244 (20·5%) ingested other organophosphorus insecticides. Patients reported ingesting from a few to several hundred mL.

Using a butyrylcholinesterase level less than 50% of the laboratory mean as a cut off, we measured substantial exposure in 218 of 240 patients (90·8%) who had taken chlorpyrifos, 106 of 136 (77·9%) who had taken dimethoate, and 47 of 57 (82·4%) who had taken fenthion. Of these patients, we detected the alleged insecticide in the plasma of 208 patients (95·5%) taking chlorpyrifos, 90 (84·9%) taking dimethoate, and 45 (95·7%) taking fenthion.

There were clear differences in human poisoning effects caused by the three insecticides (table 2) despite similar lethality in rats and classification as WHO Class II moderately hazardous pesticides.[13] Dimethoate or fenthion poisoning was more severe than chlorpyrifos poisoning. Compared with chlorpyrifos, the odds ratio (OR) of death was 3·5 (95% CI 2·2–5·4) after dimethoate and 2·2 (1·2–4·2) after fenthion.

The need for endotracheal intubation was higher with dimethoate and fenthion (table 2). Compared with chlorpyrifos, the OR for intubation was 3·1 (2·1–4·4) for dimethoate and 2·6 (1·6–4·2) for fenthion, respectively.

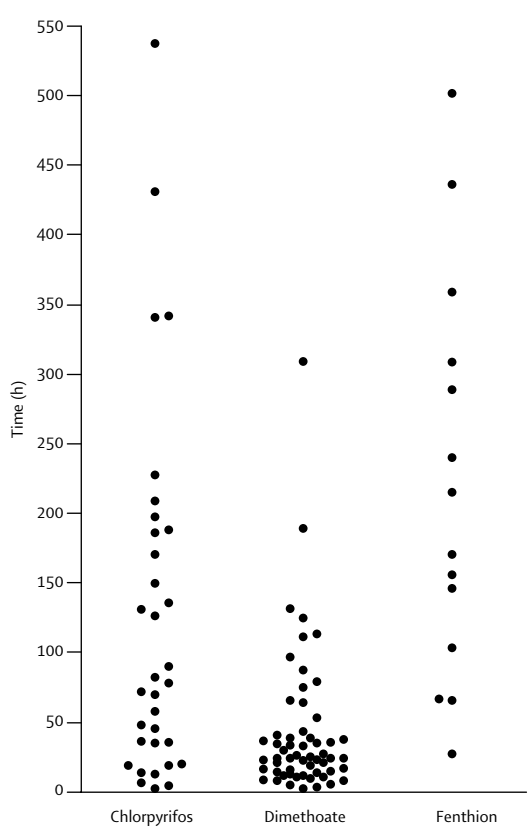

Figure 1: **Time between ingestion of insecticide and death**
Paired times of ingestion and death were available for 33 of 35 fatal chlorpyrifos cases, 60 of 61 fatal dimethoate cases, and 14 of 16 fatal fenthion cases.

Overt seizures were uncommon for all three organophosphorus insecticides (table 2).

Mode of death differed between the organophosphorus insecticides. Of the 107 cases with known times of both ingestion and death (95·5% of 112 fatal cases; figure 1), three deaths from ingesting chlorpyrifos (9% of 33) and four from dimethoate (7% of 60) were within 6 h of ingestion as a result of the acute cholinergic effects of the poisoning. By contrast, no patients poisoned by fenthion died within 24 h of ingestion. Unlike chlorpyrifos and fenthion, many deaths in patients poisoned with dimethoate (35 of 60; 58%) happened 12–48 h after ingestion from hypotensive shock (figure 1). Most presented with low Glasgow Coma Score, poor respiratory function requiring mechanical ventilation, and hypotension requiring vasopressors and atropine. Patients with fatal fenthion poisoning were often asymptomatic on admission. They initially required little atropine but ten (71% of 14) then developed cholinergic crises requiring atropine or exhibited peripheral respiratory failure (or both) and required endotracheal intubation more than 30 h after poisoning. No patients poisoned with chlorpyrifos or dimethoate with mild symptoms on admission (requiring less than 1–3 mg of atropine initially) died from delayed respiratory arrest.

Deaths from fenthion (ten of 14, 71%) or chlorpyrifos (14 of 33, 42%) were often late, after 5 days, as a result of complications of long-term ventilation or the respiratory or neurological complications of events before admission. Such late deaths were uncommon with dimethoate (four of 60, 7%).

The variability in toxic effects is unlikely to be due to differences between patients since the groups were similar. Indeed, logistic regression analysis adjusting for sex, age, recruitment to randomised controlled trial, and charcoal allocation resulted in estimates of the OR of death and intubation for dimethoate and fenthion, compared with chlorpyrifos, becoming larger.

There were clear differences on admission in the condition of patients who died (table 2). Patients with dimethoate poisoning were more deeply unconscious than those ingesting fenthion or chlorpyrifos. Seven (44%) of 16 fatal fenthion cases and six (17%) of 35 fatal chlorpyrifos cases had a normal Glasgow Coma Score on admission; only two (3%) of 61 fatal dimethoate cases had a normal score, whereas 61% had a score of 3 out of 15 on admission.

We wondered whether the variable toxicity might be due to the formulation of the organophosphorus insecticide and therefore investigated how each was prepared. We found no notable differences. Each was sold as an emulsifiable concentrate with 40–50% active ingredients (table 3). 40–50% xylene solvent was used for each insecticide; however, some chlorpyrifos and dimethoate formulators replaced part of the xylene with cyclohexanone or petroleum fractions.

	Chlorpyrifos	Dimethoate	Fenthion
WHO and EPA toxicity class	II Moderately toxic	II Moderately toxic	II Moderately toxic
Rat oral LD_{50}*			
OSHA[19]	97	250	215–245
WHO[13]	135	About 150	NG
CPH[20]	96–270	235	250
Alkyl groups	Diethyl	Dimethyl	Dimethyl
Fat solubility (log P)†	5·05	0·76	4·3
Thion or oxon	Thion	Thion	Thion
Formulation			
g/L	400	400	500
Volume (mL)	100–400	100–400	100–400
Solvents	Xylene	Xylene, or xylene and cyclohexanone	Xylene, or xylene and petroleum fractions

CPH=Crop Protection Handbook. EPA=Environmental Protection Agency. NG=not given in the source. OSHA=Occupational Safety and Health Administration, USA. *Three sources of rat oral LD_{50} values (mg/kg) given. †Log P, the logarithm of the partition coefficient between n-octanol and water, correlates with fat solubility. Values given are mean of those from two to four experimental sources.[21] Value <1·0 indicates water-soluble compound. Value >4·0 indicates a very fat-soluble compound.

Table 3: **Characteristics of the three insecticides**

Differences between the chemistry of the pesticides themselves might account for the differential toxicity. Dimethoate and fenthion are dimethyl organophosphorus insecticides, whereas chlorpyrifos is a diethyl organophosphorus insecticide (figure 2). We assessed whether variable inhibition of cholinesterases or response to oximes might explain the variable toxicity.

Considering only patients with substantial exposure (butyrylcholinesterase less than 3000 mU/mL, detectable organophosphorus insecticide), median butyrylcholinesterase activity on admission was lower after chlorpyrifos and fenthion than after dimethoate (table 1). Remarkably, despite the lesser inhibition of butyrylcholinesterase in dimethoate poisoning, the median concentration of dimethoate was much higher than that of chlorpyrifos or fenthion (table 1).

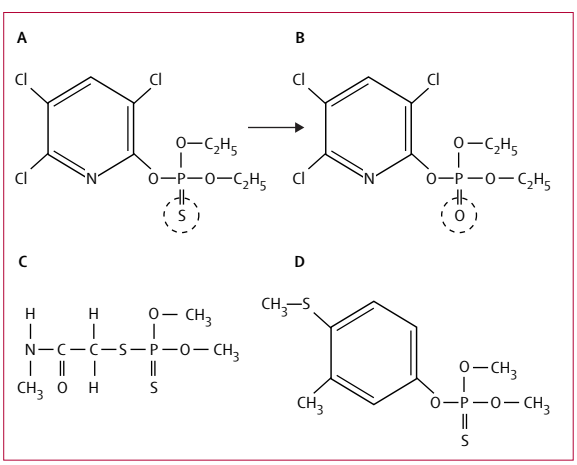

Figure 2: **Structure of the three organophosphorus pesticides**
Chlorpyrifos (A; CAS 2921-88-2) and chlorpyrifos oxon (B), the active form of chlorpyrifos after desulphuration of circled =S to =O. Dimethoate (C; CAS 60-51-5) and fenthion (D; CAS 55-38-9) must also be activated to oxon form. Note two ethyl groups attached to P in chlorpyrifos and two methyl groups attached to P in dimethoate and fenthion. Dimethoate is an aliphatic compound, chlorpyrifos and fenthion aromatic compounds.

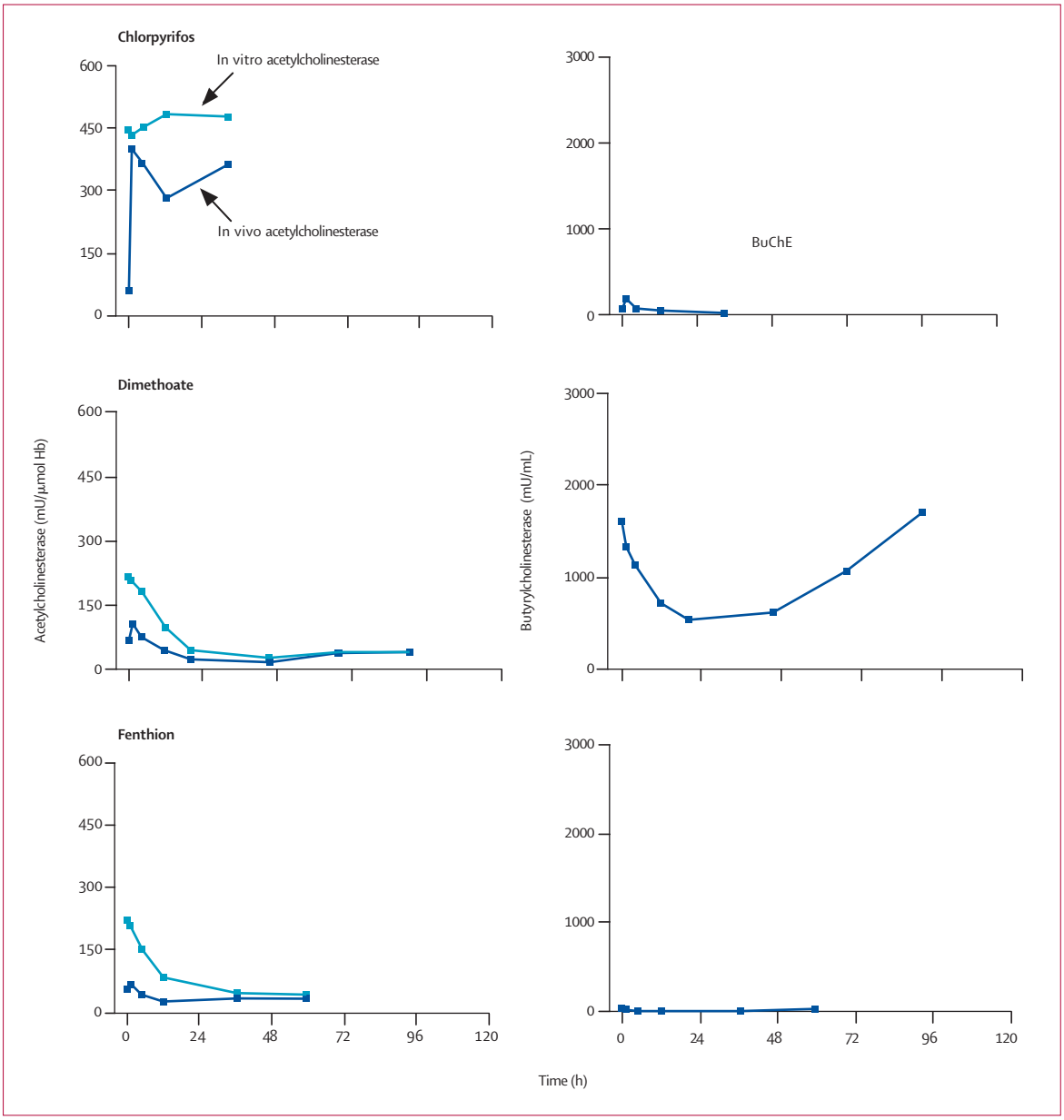

Figure 3: **Red cell acetylcholinesterase and plasma butyrylcholinesterase activities in representative cases before and after pralidoxime treatment**
Time 0=time of first pralidoxime administration. Normal acetylcholinesterase activity is around 600 mU/µmol Hb; the lower bound of normal for butyrylcholinesterase was set as 3000 mU/mL. In-vitro acetylcholinesterase assay indicates how much of inhibited acetylcholinesterase can be reactivated with supratherapeutic concentrations of oximes—ie, how much acetylcholinesterase is not yet aged and therefore responsive to oximes. Dose of oxime used in vitro is far higher than can be obtained in patients because of toxicity of oximes. Patients were chosen on basis of their similarity to median values for data. All patients followed this pattern allowing for variation due to dose and time to admission.

We analysed all 32 patients with chlorpyrifos, dimethoate, and fenthion poisoning who had less than 3000 mU/mL of butyrylcholinesterase and less than 300 mU/µmol Hb acetylcholinesterase on admission. We assayed butyrylcholinesterase, red cell acetylcholinesterase, acetylcholinesterase ageing, and plasma concentrations of organophosphorus insecticide before and after giving 1 g of pralidoxime. Median acetylcholinesterase activity on admission in these patients was similar for all three pesticides (table 2), unlike butyrylcholinesterase activity.

Response to pralidoxime differed by organophosphorus insecticide (figure 3). By 1 h, median acetylcholinesterase activity with chlorpyrifos had increased by 328 mU compared with increases of only 41 mU and 4 mU with dimethoate and fenthion (table 2). At 12 h, median acetylcholinesterase was 249 mU above admission for chlorpyrifos compared with 27 mU and 22 mU below

admission values for dimethoate and fenthion, respectively. Substantial ageing had already occurred on admission for dimethoate and fenthion compared with chlorpyrifos-inhibited acetylcholinesterase (table 2). Ageing continued for acetylcholinesterase inhibited by dimethoate or fenthion at 12 h; pralidoxime partly prevented further ageing in chlorpyrifos poisoning. Ageing was complete by 24 h in most dimethoate and fenthion cases, making oximes thereafter ineffective.

Discussion

Although the mechanism of toxicity is thought to be the same for all organophosphorus insecticides, we measured important differences in the clinical course of humans poisoned by three such compounds, despite identical treatment. We also show that the relative human toxicity of these insecticides might not be related to animal toxicity. The widely used approach of differentiating organophosphorus insecticides according to their animal LD_{50} did not accord with human toxicity, and is probably of limited value in risk assessment or management of human poisoning.

Organophosphorus insecticide self-poisoning causes hundreds of thousands of deaths each year.[3,4] Current treatment is only partly effective, with case fatality often greater than 10% in even the best intensive care units. Part of the problem is that there is little evidence on which to base management.[22] But another problem is that all organophosphorus insecticides have been grouped together, with no attempt being made to develop specific management protocols or identify particular insecticides that are difficult to treat.

Dimethoate poisoning produced a different clinical syndrome to the other organophosphorus insecticides. Some patients were deeply unconscious on admission despite having acetylcholinesterase concentrations more than 10–20% of normal. Most textbooks suggest that greater acetylcholinesterase inhibition is required for severe clinical features of poisoning. Severely poisoned patients were hypotensive on admission and died from hypotensive shock while being ventilated. The reason for this different presentation is not known. However, it may be partly due to the low fat solubility of dimethoate (table 3), causing a low volume of distribution and very high blood concentration for dimethoate.

Most fenthion deaths and many chlorpyrifos deaths occurred after several days of ventilation in intensive care. Deaths were due to complications of pesticide aspiration and hypoxic brain injury before admission or the sudden respiratory arrest of the intermediate syndrome, in addition to the complications of long-term ventilation. The rate of onset for each insecticide will determine whether respiratory arrests occur before admission or after several days in hospital.

The known toxicology of the solvents,[23] and the predominant use of xylene for all three insecticides, makes it unlikely that solvents were responsible for the variable toxicity. We were unable to find any evidence that differences in the formulations' taste and palatability might explain the differences. We did not assess the effect of acute or chronic alcohol use on organophosphorus insecticide toxicity. However, we did not note any difference in alcohol use that might account for the variable toxicity for the three insecticides.

In the absence of conclusive clinical trial data, there has been extensive debate about the effectiveness of oximes as treatment for organophosphorus insecticide poisoning.[12,24] Asian doctors have reported no benefit from pralidoxime;[25,26] however, a 250-mg bolus of obidoxime (equivalent to about 2 g pralidoxime) clearly reactivates acetylcholinesterase inhibited by the dimethyl organophosphorus insecticide parathion.[12,27] We found that patients poisoned by a diethyl organophosphorus insecticide (chlorpyrifos) responded well to pralidoxime, whereas those poisoned by two dimethyl organophosphorus insecticides (dimethoate, fenthion) responded poorly. This finding suggests that uncertainty about oxime effectiveness is likely to be due to confounding from studying these insecticides as a group rather than as individual compounds.

The dose of pralidoxime used in this cohort was lower than the current WHO recommended dose.[28] We do not think that this factor was responsible for its poor efficacy in dimethoate or fenthion poisoning—250 mg obidoxime also has a poor effect in dimethoate poisoning, with complete ageing within 20 h (figure 2, B in reference 12). The failure of pralidoxime to reactivate dimethoate-inhibited acetylcholinesterase was not due to its high blood concentration since a similar failure occurred with fenthion at a blood concentration 100 times lower.

The low dose of pralidoxime was probably sub-optimum for chlorpyrifos poisoning, allowing some acetylcholinesterase to become reinhibited and aged after the initial response. High-dose oxime was effective at obtaining sustained acetylcholinesterase reactivation and slowing ageing with parathion (figure 2, D in reference 12). However, the use of low doses of pralidoxime does not explain the variable toxicity. Higher doses might have further decreased the toxic effects and mortality of chlorpyrifos poisoning, but are unlikely to have greatly benefited patients poisoned by fenthion or dimethoate, especially those with high-dose poisoning.[11,12]

Butyrylcholinesterase activity on admission cannot be used to predict outcome or severity unless the organophosphorus insecticide is known. The degree of inhibition of butyrylcholinesterase on admission varied by insecticide: the activity was zero for many symptomatic chlorpyrifos and fenthion cases but more than 20% of normal for some severe dimethoate cases.

A limitation of this study is that a blood sample was not available from all patients to identify the pesticide

ingested. However, samples were available for 54% of patients, and, in those with substantial exposure, the reported insecticide was detected in 85–95% of patients, suggesting that the history effectively identified the ingested compound. We did not exclude patients without detectable pesticide in the blood since the lack of blood samples for some patients would have introduced bias. A further limitation is that acetylcholinesterase values were available for very few patients. However, the clear difference in response to pralidoxime in this small sample suggests the finding is likely to be robust; more patients are now being studied.

This finding of significant clinical differences between organophosphorus insecticides is important for pesticide regulation and clinical trials. Previously, regulatory decisions have sometimes been based on the WHO classification by animal toxicity.[14] However, if these findings can be generalised to all dimethyl or diethyl organophosphorus insecticides, it may be safer to allow the agricultural use of slowly activated diethyl organophosphorus insecticides, which respond well to oximes, rather than the use of dimethyl organophosphorus insecticides that are difficult to treat, irrespective of their animal toxicity.

Earlier trials of pralidoxime are confounded by the presence of both dimethyl and diethyl organophosphorus insecticides, some of which might not respond to oximes.[12] Future trials will need to identify the exact pesticide taken by each patient. Pralidoxime was not efficacious in reactivating acetylcholinesterase inhibited by the dimethyl pesticides dimethoate and fenthion. More research is needed to determine whether this poor response to oximes is a general property of dimethyl organophosphorus insecticides. Possible public health responses include banning organophosphorus insecticides that do not respond to oximes[29] and developing new therapies that allow oximes to work better.

Finally, management guidelines for organophosphorus poisoning do not differentiate between individual pesticides. Our findings suggest that it is not adequate to consider such poisoning as a homogeneous entity. The variable clinical syndromes and response to oximes suggest that future studies could lay the groundwork for developing specific management protocols for individual organophosphorus pesticides.

Contributors

M Eddleston designed and set up the cohort, designed this study, did the analysis and wrote the first draft of the report. P Eyer, F Worek, L von Meyer, and L Szinicz analysed blood and pesticide samples. F Mohamed and L Senarathna ran the trial centres and, with M Eddleston, extracted and checked patients' data for analysis. E Juszczak helped design the trial and contributed to the statistics for this paper. A Hittarage, S Azhar, and W Dissanayake had clinical responsibility for patients. M H R Sheriff and A H Dawson organised the cohort through the South Asian Clinical Toxicology Research Collaboration. N A Buckley helped design the cohort study and contributed to the analysis. All authors helped improve the study design and finalising the report.

Conflict of interest statement

We declare that we have no conflict of interest.

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E X T O X N E T

Extension Toxicology Network

Pesticide Information Profiles

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Revised June 1996

Chlorpyrifos

Trade and Other Names: Trade names include Brodan, Detmol UA, Dowco 179, Dursban, Empire, Eradex, Lorsban, Paqeant, Piridane, Scout, and Stipend.

Regulatory Status: The EPA has established a 24-hour reentry interval for crop areas treated with emulsifiable concentrate or wettable powder formulations of chlorpyrifos unless workers wear protective clothing. Chlorpyrifos is toxicity class II - moderately toxic. Products containing chlorpyrifos bear the Signal Word WARNING or CAUTION, depending on the toxicity of the formulation. It is classified as a General Use Pesticide (GUP).

Chemical Class: organophosphate

Introduction: Chlorpyrifos is a broad-spectrum organophosphate insecticide. While originally used primarily to kill mosquitoes, it is no longer registered for this use. Chlorpyrifos is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings,

farm buildings, storage bins, and commercial establishments. Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison. It is available as granules, wettable powder, dustable powder and emulsifiable concentrate.

Formulation: It is available as granules, wettable powder, dustable powder, and emulsifiable concentrate.

Toxicological Effects:

- **Acute toxicity:** Chlorpyrifos is moderately toxic to humans [43]. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant [2]. While some organophosphates are readily absorbed through the skin, studies in humans suggest that skin absorption of chlorpyrifos is limited [2]. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, cholinesterase impairment, or liver malfunction are at increased risk from exposure to chlorpyrifos. Some organophosphates may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure which may or may not have produced immediate symptoms [2]. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, and in some cases residual impairment will remain [2]. Plasma cholinesterase levels activity have been shown to be inhibited when chlorpyrifos particles are inhaled [8]. The oral LD50 for chlorpyrifos in rats is 95 to 270 mg/kg [2,13]. The LD50 for chlorpyrifos is 60 mg/kg in mice, 1000 mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep [2,13,44]. The dermal LD50 is greater than 2000 mg/kg in rats, and 1000 to 2000 mg/kg in rabbits [2,13,45]. The 4-hour inhalation LC50 for chlorpyrifos in rats is greater than 0.2 mg/L [46].
- **Chronic toxicity:** Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and drowsiness or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported [8]. When technical chlorpyrifos was fed to dogs for 2 years, increased liver weight occurred at 3.0 mg/kg/day. Signs of cholinesterase inhibition occurred at 1 mg/kg/day. Rats and mice given technical chlorpyrifos in the diet for 104 weeks showed no adverse effects other than cholinesterase inhibition [43]. Two-year feeding studies using doses of 1 and 3 mg/kg/day of chlorpyrifos in rats showed moderate depression of cholinesterase. Cholinesterase levels recovered when the experimental feeding was discontinued [2]. Identical results occurred in a 2-year feeding study with dogs. No long term health effects were seen in either the dog or rat study [2,47]. A measurable change in plasma and red blood cell cholinesterase levels was seen in workers exposed to chlorpyrifos spray. Human volunteers who ingested 0.1 mg/kg/day of chlorpyrifos for 4 weeks showed significant plasma cholinesterase inhibition [47].
- **Reproductive effects:** Current evidence indicates that chlorpyrifos does not adversely affect reproduction. In two studies, no effects were seen in animals tested at dose levels up to 1.2 mg/kg/day [8]. No effects on reproduction occurred in a three-generation study with rats fed dietary doses as high as 1 mg/kg/day [43,47]. In another study in which rats were fed 1.0 mg/kg/day for two generations, the only effect observed was a slight increase in the number of deaths of newborn offspring [2].
- **Teratogenic effects:** Available evidence suggests that chorpyrifos is not teratogenic. No teratogenic effects in offspring were found when pregnant rats were fed doses as high as 15 mg/kg/day for 10 days. When pregnant mice were given doses of 25 mg/kg/day for 10 days, minor skeletal variations and a decrease in fetal length occurred [43,45]. No birth defects were seen in the offspring of male and female rats fed 1.0 mg/kg/day during a three-generation reproduction and fertility study [2,47].
- **Mutagenic effects:** There is no evidence that chlorpyrifos is mutagenic. No evidence of mutagenicity was found in any of four tests performed [43].

- **Carcinogenic effects:** There is no evidence that chlorpyrifos is carcinogenic. There was no increase in the incidence of tumors when rats were fed 10 mg/kg/day for 104 weeks, nor when mice were fed 2.25 mg/kg/day for 105 weeks [43].
- **Organ toxicity:** Chlorpyrifos primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning.
- **Fate in humans and animals:** Chlorpyrifos is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure [8]. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly [2]. After a single oral dose, the half-life of chlorpyrifos in the blood appears to be about 1 day [41]. Chlorpyrifos is eliminated primarily through the kidneys [8]. Following oral intake of chlorpyrifos by rats, 90% is removed in the urine and 10% is excreted in the feces [13]. It is detoxified quickly in rats, dogs, and other animals [8]. The major metabolite found in rat urine after a single oral dose is trichloropyridinol (TCP). TCP does not inhibit cholinesterase and it is not mutagenic [8]. Chlorpyrifos does not have a significant bioaccumulation potential [8]. Following intake, a portion is stored in fat tissues but it is eliminated in humans, with a half-life of about 62 hours [2]. When chlorpyrifos (Dursban) was fed to cows, unchanged pesticide was found in the feces, but not in the urine or milk [48]. However, it was detected in the milk of cows for 4 days following spray dipping with a 0.15% emulsion. The maximum concentration in the milk was 0.304 ppm [2]. In a rat study, chlorpyrifos did not accumulate in any tissue except fat [49].

Ecological Effects:

- **Effects on birds:** Chlorpyrifos is moderately to very highly toxic to birds [43]. Its oral LD50 is 8.41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows, and 32 mg/kg in chickens [8,13,43]. The LD50 for a granular product (15G) in bobwhite quail is 108 mg/kg [13,43]. At 125 ppm, mallards laid significantly fewer eggs [43]. There was no evidence of changes in weight gain, or in the number, weight, and quality of eggs produced by hens fed dietary levels of 50 ppm of chlorpyrifos [8].
- **Effects on aquatic organisms**: Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms [43]. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths [43]. Chlorpyrifos toxicity to fish may be related to water temperature. The 96-hour LC50 for chlorpyrifos is 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow [50]. When fathead minnows were exposed to Dursban for a 200-day period during which they reproduced, the first generation of offspring had decreased survival and growth, as well as a significant number of deformities. This occurred at approximately 0.002 mg/L exposure for a 30-day period [8]. Chlorpyrifos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through fry stages have shown bioconcentration values of 58 to 5100 [51]. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers [52]. Smaller organisms appear to be more sensitive than larger ones [50].
- **Effects on other organisms:** Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to wildlife and honeybees [13,48].

Environmental Fate:

- **Breakdown in soil and groundwater:** Chlorpyrifos is moderately persistent in soils. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions [12,19]. The soil half-life of chlorpyrifos was from 11 to 141 days in seven soils ranging in texture from loamy sand to clay and with soil pHs from 5.4 to 7.4. Chlorpyrifos was less persistent in the soils with a higher pH [51]. Soil half-life was not affected by soil texture or organic matter content. In anaerobic soils, the half-life was 15 days in loam and 58 days in clay soil [43]. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. When applied to moist soils, the volatility half-life of chlorpyrifos was 45 to 163 hours, with 62 to 89% of the applied chlorpyrifos remaining on the soil after 36 hours [51]. In another study, 2.6 and 9.3% of the chlorpyrifos applied to sand or silt loam soil remained after 30 days [51]. Chlorpyrifos

adsorbs strongly to soil particles and it is not readily soluble in water [19,51]. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater [51]. TCP, the principal metabolite of chlorpyrifos, adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils [43].

- **Breakdown in water:** The concentration and persistence of chlorpyrifos in water will vary depending on the type of formulation. For example, a large increase in chlorpyrifos concentrations occurs when emulsifiable concentrations and wettable powders are released into water. As the pesticide adheres to sediments and suspended organic matter, concentrations rapidly decline. The increase in the concentration of insecticide is not as rapid for granules and controlled release formulations in the water, but the resulting concentration persists longer [50]. Volatilization is probably the primary route of loss of chlorpyrifos from water. Volatility half-lives of 3.5 and 20 days have been estimated for pond water [51]. The photolysis half-life of chlorpyrifos is 3 to 4 weeks during midsummer in the U.S. Its change into other natural forms is slow [52]. Research suggests that this insecticide is unstable in water, and the rate at which it is hydrolyzed increases with temperature, decreasing by 2.5- to 3-fold with each 10 C drop in temperature. The rate of hydrolysis is constant in acidic to neutral waters, but increases in alkaline waters. In water at pH 7.0 and 25 C, it had a half-life of 35 to 78 days [12].
- **Breakdown in vegetation:** Chlorpyrifos may be toxic to some plants, such as lettuce [36]. Residues remain on plant surfaces for approximately 10 to 14 days. Data indicate that this insecticide and its soil metabolites can accumulate in certain crops [8].

Physical Properties:

- **Appearance:** Technical chlorpyrifos is an amber to white crystalline solid with a mild sulfur odor [13].
- **Chemical Name:** O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate [13]
- **CAS Number:** 2921-88-2
- **Molecular Weight:** 350.62
- **Water Solubility:** 2 mg/L @ 25 C [13]
- **Solubility in Other Solvents:** benzene s.; acetone s.; chloroform s.; carbon disulfide s.; diethyl ether s.; xylene s.; methylene chloride s.; methanol s. [13]
- **Melting Point:** 41.5-44 C [13]
- **Vapor Pressure:** 2.5 mPa @ 25 C [13]
- **Partition Coefficient:** 4.6990 [13]
- **Adsorption Coefficient:** 6070 [19]

Exposure Guidelines:

- **ADI:** 0.01 mg/kg/day [38]
- **MCL**: Not Available
- **RfD:** 0.003 mg/kg/day [53]
- **PEL:** 0.2 mg/m3 (8-hour) (skin)
- **HA**: 0.02 mg/L (lifetime) [53]
- **TLV**: Not Available

Basic Manufacturer:

DowElanco
9330 Zionsville Rd.
Indianapolis, IN 46268-1054

- **Phone:** 317-337-7344
- **Emergency:** 800-258-3033

References:

References for the information in this PIP can be found in Reference List Number 5

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